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A MANUAL

OF

CLINICAL DIAGNOSIS

BY MEANS OF MICROSCOPICAL AND CHEMICAL METHODS,

FOR

STUDENTS, HOSPITAL PHYSICIANS, AND PRACTITIONERS.

BY

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Of Baltimore, Md.

FIFTH EDITION, THOROUGHLY REVISED AND ENLARGED.

ILLUSTRATED WITH 150 ENGRAVINGS AND 22 PLATES IN COLORS.

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TO

MY WIFE,

WHO HAS SO FAITHFULLY AIDED IN ITS PREPARATION,

THIS EDITION ALSO

IS

AFFECTIONATELY DEDICATED.

PREFACE TO THE FIFTH EDITION.

THE demand for a new edition has been construed by the author not only as an expression of professional favor, but also and more particularly as still another opportunity of keeping the book abreast of a most active and important branch of medicine. Exact methods of diagnosis are of comparatively recent development, and as they necessarily underlie successful therapeusis, they should be a part of the equipment of every physician. They are not recondite or abstruse, but on the contrary are susceptible of plain statement and direct application. Such has been the object of this work from its inception, namely, to simplify the physician's work and to increase its efficiency by enabling him to eliminate doubt from his diagnosis. The book has been adapted to the needs of students as well as of graduates, in view of the fact that the subject is already included in the curricula of many colleges, and is destined to be required in all. The author's endeavor has been to state the best methods clearly and simply, with all necessary instructions, and to render the book as modern and practical as possible.

Besides a careful revision, this edition embodies much new matter which has appeared in the literature of the past two years. The chapter on the blood has been almost entirely rewritten, and has been enlarged by sixty pages. Special pains have been taken with the chapter on technique. A section dealing with the nature of anilin dyes and the principles of staining has been introduced, which it is hoped will render this portion of the book more interesting to the clinical laboratory worker, and will serve as a guide to further investigation. For convenience of reference, the subject of leucocytosis has been rearranged in such manner that hyperleucocytosis and hypoleucocytosis are separately considered in connection with the different varieties of leucocytes. A new section deals with the kryoscopic examination of the blood. The bacteriology and parasitology of the blood have been enlarged, with

sections on paratyphoid fever, gonococcus septicæmia, bubonic plague, trypanosomiasis, and spotted fever. Material changes and additions have furthermore been made in the chapters on the feces, the sputum, the urine, and on transudates and exudates, and minor alterations occur throughout the book. Illustrations have been added wherever they appeared necessary to elucidate the text.

To the profession at large I am indebted for the kind reception which has been accorded to the Diagnosis. To Messrs. Lea Brothers & Co. I wish to express my appreciation of their many acts of courtesy and liberality. The six new colored plates (Nos. I., II., III., IV., VI., and XXI.) I owe to my wife, and thank her at this place also for the many hours of patient study which she has spent in my laboratory for the purpose of familiarizing herself with blood morphology more particularly, so as to be able to produce illustrations which should be more nearly true to nature than any that have hitherto been prepared. To what extent she has succeeded I leave to the laboratory student to judge.

CHARLES E. SIMON.

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January, 1904.

PREFACE TO THE FIRST EDITION.

It is curious to note that, notwithstanding the great importance of clinical chemistry and microscopy, but little attention is paid to these subjects, either by hospital physicians or by those engaged in general practice. This lack of interest is referable primarily to the fact that a systematic study of these branches has hitherto been greatly neglected, not only in American medical schools, but also in those of Europe.

It is no rarity to hear physicians in general practice claim that they are too busy to conduct careful examinations of the urine, sputum, blood, gastric juice, etc. Would it not be reasonable to suppose, however, that a physician who is overwhelmed with work to such an extent that he cannot find the time to make use of aids in diagnosis which are quite as important as the stethoscope, the laryngoscope, or the ophthalmoscope, would be in a position to employ an assistant in his laboratory? The younger practitioner is certainly not placed in such a dilemma, and it is a fair assumption that he could successfully compete with his more experienced colleague, in matters of diagnosis at least, were he to familiarize himself sufficiently with laboratory methods of diagnosis.

The time is at hand when the practice of medicine is becoming what it was long ago, but then unjustly, called, a true science and art. No continuing success can be built on empiricism or upon the proportion of guesswork which is inseparable from dependence upon "the experienced eye." "Diagnosis" is now the password in medical science. A knowledge of electro-diagnosis, of ophthalmoscopy, of laryngoscopy, etc., is at the present day a *sine qua non* for accurate diagnosis. Equally important at all times, and frequently even more important, is a knowledge of clinical chemistry and microscopy. It is inconceivable that a physician can rationally diagnosticate and treat diseases of the stomach, intestines, kidneys, and liver, etc., without laboratory facilities.

It has been the author's aim to present to students and physicians those facts in clinical chemistry and microscopy which are of practical importance. With the hope of exciting interest in these unjustly neglected subjects, he has not confined himself to bare statements of facts, which must in themselves be dry and uninteresting, but he has attempted to point out the reasons which have led up to the conclusions reached.

Chemical and microscopical methods are described in detail, so that the student and practitioner who has not had special training in such manipulations will be enabled to obtain satisfactory results.

The subject-matter covers the examination of the blood, the secretions of the mouth, the gastric juice, feces, nasal secretion, sputum, urine, transudates, exudates, cystic contents, semen, vaginal discharges, and milk. In every case a description of normal material precedes the pathological considerations, which latter in turn are followed by an account of the methods used in examination. A glance at the table of contents will furnish an idea of the various subjects considered under each heading.

In conclusion, it is the agreeable duty of the author to express his sincerest thanks to his wife for assistance without which this volume could not have been written, and likewise for those illustrations which are original; to Dr. William H. Welch for his kindness in placing the former Hygienic Laboratory of the Johns Hopkins Hospital at his disposal during the years 1892 and 1893; to Dr. W. Milton Lewis for much valuable aid in the correction of the manuscript and proof-sheets; and to Messrs. Lea Brothers & Co. for the typographical excellence of the work, the extremely satisfactory reproduction of the drawings, and for many acts of courtesy.

CHARLES E. SIMON.

Baltimore, Md., 1896.

CONTENTS.

CHAPTER I.

THE BLOOD.

PAGE

General considerations 17
General characteristics of the blood 17
color 17
odor 18
specific gravity 18
determination according to Roy 18
determination according to Hammerschlag 19
determination according to Schmaltz and Peiper 19
indirect estimation of the hæmoglobin 20
estimation of the solids of the blood 20
reaction 21
estimation of the alkalinity according to Landois–v. Jaksch 22
estimation of the alkalinity according to Löwy 24
estimation of the alkalinity according to Engel 24
estimation of the alkalinity according to Dare 25
Chemical examination of the blood 27
general chemistry of the blood 27
coagulation of the blood 29
blood-pigments 32
hæmoglobin and oxyhæmoglobin 32
hæmoglobinæmia 36
carbon monoxide hæmoglobin 37
nitric oxide hæmoglobin 38
hydrogen sulphide hæmoglobin 38
carbon dioxide hæmoglobin 38
hæmatin 38
hæmin 39
methæmoglobin 40
hæmatoidin 40
hæmatoporphyrin 41
the spectroscope 41
the proteids of the blood 43
the carbohydrates 44
sugar 44
estimation of the sugar in the blood 45
Williamson's diabetic blood test 46
glycogen 46
cellulose 47
urea 47
uræmia 47
ammonia 47
uric acid and xanthin-bases 48
fat and fatty acids 50
lactic acid 52

Chemical examination of the blood—*Continued.* PAGE
biliary constituents 53
acetone 54
Microscopical examination of the blood 54
the red corpuscles 54
variations in the size of the red corpuscles 54
variations in the form of the red corpuscles 54
variations in the color of the red corpuscles and color index 56
variations in the number of the red corpuscles 58
behavior toward anilin dyes (polychromatophilia) 62
granular degeneration 65
Cabot's ring bodies 67
Ehrlich's hæmoglobinæmic Innenkörper 67
nucleated red corpuscles 67
the leucocytes 71
general differentiation of the various forms of leucocytes 71
differentiation of the leucocytes according to their behavior toward anilin dyes 73
the small mononuclear leucocytes 73
the large mononuclear leucocytes 75
the polynuclear neutrophilic leucocytes 77
the polynuclear eosinophilic leucocytes 79
the mast cells 80
the myelocytes 81
Ehrlich's pseudolymphocytes 83
Türk's irritation forms 83
iodophilia 83
leucocytosis 85
polynuclear neutrophilic hyperleucocytosis 86
polynuclear neutrophilic hypoleucocytosis 96
polynuclear eosinophilic hyperleucocytosis 100
polynuclear eosinophilic hypoleucocytosis 104
lymphocytosis 105
lymphopenia 107
variations in number of the large mononuclear leucocytes 107
variations in number of the mast cells 108
myelocytosis 109
the plaques 112
the dust particles of Müller 114
General technique 114
slides and cover-glasses 114
the blood mount 115
fixation 118
The anilin dyes and principles of staining 119
Methods of staining 123
the eosinate of methylene-blue 123
Ehrlich's triacid stain 124
Pappenheim's triacid stains 126
the Romanowsky methods 127
method of Nocht 127

Methods of staining—*Continued.* PAGE
method of Michaelis and Wolff . . . 129
method of Giemsa . . . 129
method of Reuter . . . 129
method of Leishman . . . 130
method of Wright . . . 130
method of Koch . . . 132
method of Ewing . . . 132
method of Ziemann . . . 133
Ehrlich's eosin-methylene-blue-methylal . . . 133
Michaelis' eosin-methylene-blue-acetone . . . 134
Strauss and Rohnstein's method . . . 134
staining with eosin . . . 135
staining with Ehrlich's triglycerin mixture . . . 135
staining with Ehrlich's neutral mixture . . . 135
staining with Ehrlich's methyl-green-fuchsin . . . 136
staining wth Pappenheim's-methyl-green pyronin . . . 136
staining with Ehrlich's dahlia . . . 136
Westphal's method . . . 136
the Chenzinsky-Plehn methods . . . 137
Futcher's carbol-thionin . . . 137
Ehrlich's hæmatoxylin-eosin . . . 138
demonstration of iodophilia . . . 138
distribution of alkali . . . 139
Enumeration of the corpuscles of the blood . . . 139
method of Thoma-Zeiss . . . 139
enumeration of the red cells . . . 140
enumeration of the leucocytes . . . 143
differential leucocyte counting . . . 146
enumeration of the plaques . . . 147
The hæmatokrit . . . 147
Estimation of hæmoglobin . . . 150
Dare's hæmoglobinometer . . . 151
Fleischl's hæmoglobinometer . . . 153
Oliver's hæmoglobinometer . . . 155
Gowers' hæmoglobinometer . . . 157
Talquist's method . . . 158
Estimation of blood-iron . . . 158
Kryoscopic examination of the blood . . . 163
Bacteriology and parasitology of the blood . . . 165
typhoid fever . . . 165
Widal's serum test . . . 165
paratyphoid fever . . . 170
pneumonia . . . 171
sepsis . . . 173
gonococcus septicæmia . . . 176
anthrax . . . 177
acute miliary tuberculosis . . . 177
glanders . . . 178
influenza . . . 178

Bacteriology and parasitology of the blood—*Continued.* PAGE
relapsing fever . . . 179
Malta fever . . . 180
yellow fever . . . 180
bubonic plague . . . 181
malaria . . . 182
trypanosomiasis . . . 191
spotted fever (tick fever) . . . 192
filariasis . . . 192
distomiasis (bilharziasis) . . . 195
anguilluliasis . . . 197

CHAPTER II.

THE SECRETIONS OF THE MOUTH.

Saliva . . . 198
general characteristics . . . 198
chemistry of the saliva . . . 198
microscopical examination of the saliva . . . 200
pathological alterations . . . 202
Special diseases of the mouth . . . 203
tuberculosis of the mouth . . . 203
actinomycosis . . . 203
catarrhal stomatitis . . . 203
ulcerative stomatitis . . . 203
gonorrhœal stomatitis . . . 203
thrush . . . 204
Tartar . . . 204
Coating of the tongue . . . 204
Coating of the tonsils . . . 205
pharyngomycosis leptothrica . . . 205
tonsillitis . . . 205
glandular fever . . . 206
diphtheria . . . 206
scarlatina . . . 209

CHAPTER III.

THE GASTRIC JUICE AND THE GASTRIC CONTENTS.

PAGE
The secretion of gastric juice . . . 210
Test-meals . . . 211
the test-breakfast of Ewald and Boas . . . 211
the test-dinner of Riegel . . . 212
the double test-meal of Salzer . . . 212
the test-breakfast of Boas . . . 212
The stomach-tube . . . 212
contraindications to the use of the tube . . . 213
introduction of the tube . . . 213

PAGE
General characteristics of the gastric juice . . . 215
amount . . . 215
Chemical examination of the gastric juice . . . 216
chemical composition of the gastric juice . . . 216
the acidity of the gastric juice . . . 217
determination of the acidity of the gastric juice . . . 219
source of the hydrochloric acid . . . 221
significance of free hydrochloric acid . . . 222
the amount of free hydrochloric acid . . . 224
euchlorhydria . . . 224
hypochlorhydria . . . 224
anachlorhydria . . . 224
hyperchlorhydria . . . 225
test for free acids . . . 225
test for free hydrochloric acid . . . 226
the dimethyl-amido-azo-benzol test . . . 226
the phloroglucin-vanillin test . . . 226
the resorcin test . . . 227
the tropæolin test . . . 228
Mohr's test . . . 228
the benzopurpurin test . . . 228
the combined hydrochloric acid . . . 229
quantitative estimation of the hydrochloric acid . . . 230
Töpfer's method . . . 230
Sahli's method . . . 232
Martius and Lüttke's method . . . 232
Leo's method . . . 235
the ferments of the gastric juice and their zymogens . . . 236
pepsin and pepsinogen . . . 236
tests for pepsin and pepsinogen . . . 238
quantitative estimation . . . 238
chymosin and chymosinogen . . . 239
tests for chymosin and chymosinogen . . . 240
quantitative estimation . . . 241
the products of gastric digestion . . . 241
digestion of the native albumins . . . 241
digestion of the proteids . . . 242
digestion of the albuminoids . . . 242
digestion of the carbohydrates . . . 242
analysis of the products of albuminous digestion . . . 244
tests for the products of carbohydrate digestion . . . 245
lactic acid . . . 245
mode of formation and clinical significance . . . 245
tests for lactic acid . . . 248
Uffelmann's test . . . 248
Kelling's test . . . 249
Strauss' test . . . 249
Boas' test . . . 250
quantitative estimation of lactic acid according to Boas' method . . . 251
the fatty acids . . . 253

Chemical examination of the gastric juice—*Continued.* PAGE
mode of formation and clinical significance . . . 253
tests for butyric acid . . . 254
tests for acetic acid . . . 255
quantitative estimation of the fatty acids . . . 255
quantitative estimation of the organic acids . . . 255
gases . . . 256
acetone . . . 258
ptomaïns and toxalbumins . . . 258
vomited material . . . 259
food-material . . . 259
mucus . . . 260
gastrosuccorrhœa mucosa . . . 260
saliva . . . 261
bile . . . 261
pancreatic juice . . . 261
blood . . . 261
test of Müller and Weber . . . 261
Donogany's method . . . 262
pus . . . 262
stercoraceous material . . . 262
parasites . . . 263
odor . . . 263
Microscopical examination of the gastric contents . . . 264
the Boas-Oppler bacillus . . . 264
sarcinæ . . . 264
shreds of mucous membrane . . . 265
tumor particles . . . 266
Examination of the motor power of the stomach . . . 266
Leube's method . . . 267
the salol test of Ewald and Sievers . . . 267
Examination of the resorptive power of the stomach . . . 268
Indirect examination of the gastric juice . . . 268
Günzburg's method . . . 268
Simon's method . . . 269

CHAPTER IV.

THE FECES.

PAGE
Examination of normal feces . . . 270
general characteristics . . . 270
number of stools . . . 270
amount . . . 270
consistence and form . . . 271
odor . . . 271
color . . . 271
macroscopical constituents . . . 272
alimentary detritus . . . 272
foreign bodies . . . 272
microscopical constituents . . . 272

Examination of normal feces—*Continued.* PAGE
constituents derived from food . . . 272
morphological elements derived from the alimentary canal . . . 274
crystals . . . 274
parasites . . . 275
vegetable parasites . . . 275
fungi . . . 276
schizomycetes . . . 276
bacteria . . . 276
chemistry of normal feces . . . 278
reaction . . . 278
general composition . . . 278
phenol, indol, and skatol . . . 279
fatty acids . . . 280
cholesterin . . . 282
the biliary acids . . . 283
pigments . . . 283
xanthin bases . . . 285
Pathology of the feces . . . 285
general characteristics . . . 285
number of stools . . . 285
consistence and form . . . 285
amount . . . 286
odor . . . 286
reaction . . . 286
color . . . 287
macroscopical constituents . . . 289
alimentary constituents . . . 289
mucus and mucous cylinders . . . 290
biliary and intestinal concretions . . . 291
analysis of gall-stones . . . 292
coproliths . . . 292
intestinal sand . . . 292
microscopical examination . . . 293
technique . . . 293
remnants of food . . . 293
Schmidt's fermentation test . . . 293
Leiner's test for casein . . . 295
epithelium . . . 295
red blood-corpuscles . . . 296
mucus . . . 296
leucocytes . . . 296
crystals . . . 297
animal parasites . . . 297
protozoa . . . 298
Amœba coli . . . 299
Cercomonas hominis . . . 301
Trichomonas intestinalis . . . 302
Megastoma entericum . . . 303
Balantidium coli . . . 304
vermes . . . 305
Tænia saginata . . . 306

Pathology of the feces—*Continued.* PAGE
Tænia solium . . . 307
Tænia nana . . . 308
Tænia diminuta . . . 309
Tænia cucumerina . . . 309
Tænia africana . . . 310
Tænia madagascariensis . . . 311
Bothriocephalus latus . . . 311
Krabbea grandis . . . 313
Distoma hepaticum . . . 313
Distoma lanceolatum . . . 314
Distoma Buskii . . . 314
Distoma sibiricum . . . 314
Distoma spatulatum . . . 314
Distoma conjunctum . . . 315
Distoma heterophyes . . . 315
Amphistomum hominis . . . 315
Ascaris lumbricoides . . . 315
Ascaris mystax . . . 316
Ascaris maritima . . . 316
Oxyuris vermicularis . . . 316
Anchylostomum duodenale . . . 317
Trichocephalus hominis . . . 320
Trichina spiralis . . . 320
Anguillula intestinalis . . . 320
insecta . . . 323
vegetable parasites . . . 323
bacillus of cholera . . . 323
Finkler-Prior bacillus . . . 325
typhoid bacillus . . . 325
tubercle bacillus . . . 328
Bacillus coli communis . . . 328
Bacillus lactis aërogenes . . . 328
Bacillus pyocyaneus . . . 329
Bacillus acidophilus . . . 329
Proteus vulgaris . . . 330
Bacillus dysenteriæ . . . 331
Chemistry of the feces . . . 333
ptomaïns . . . 334
Meconium . . . 334

CHAPTER V.

THE NASAL SECRETION.

Physiology and pathology of the nasal secretion . . . 336

CHAPTER VI.

THE SPUTUM.

PAGE

General technique 338
General characteristics of the sputa 339
amount 339
consistence 339
color 340
odor 340
specific gravity 341
configuration of sputa 341
Macroscopical constituents of sputum 342
elastic tissue 342
fibrinous casts 342
Curschmann's spirals 344
echinococcus membranes 346
concretions 346
foreign bodies 346
Microscopical examination 346
leucocytes 346
red blood-corpuscles 348
epithelial cells 348
elastic tissue 350
animal parasites 352
Tænia echinococcus 352
Trichomonades 353
Amœba coli 353
Distoma pulmonale 353
Distoma hæmatobium 354
vegetable parasites 354
pathogenic organisms 354
the tubercle bacillus 354
methods of staining 357
Pappenheim's method 357
Gabett's method 357
Weigert-Ehrlich's method 358
Ziehl-Neelsen's method 358
cultivation of tubercle bacillus 359
the Diplococcus pneumoniæ 360
the bacillus of influenza 360
the bacillus of whooping-cough 361
the smegma bacillus 361
the typhoid bacillus 362
the plague bacillus 362
actinomycosis 362
non-pathogenic organisms 363
Oïdium albicans 363
Sarcina pulmonalis 363
crystals 363
Charcot-Leyden crystals 364

Microscopical examination—*Continued.* PAGE
hæmatoidin . . . 364
cholesterin . . . 365
fatty acid crystals . . . 365
leucin and tyrosin . . . 365
calcium oxalate . . . 365
triple phosphates . . . 365
the pneumoconioses . . . 365
anthracosis . . . 365
siderosis . . . 365
chalicosis . . . 366
stycosis . . . 366
Chemistry of the sputum . . . 366

CHAPTER VII.

THE URINE.

General characteristics of the urine . . . 369
general appearance . . . 369
color . . . 370
odor . . . 371
consistence . . . 371
quantity . . . 371
polyuria . . . 372
oliguria . . . 375
specific gravity . . . 375
determination of the specific gravity . . . 378
determination of the solid constituents . . . 380
Reaction . . . 380
determination of the acidity of the urine . . . 384
Freund's method . . . 384
Chemistry of the urine . . . 385
general chemical composition of the urine . . . 385
quantitative estimation of the mineral ash of the urine . . . 386
the chlorides . . . 387
test for chlorides in the urine . . . 390
quantitative estimation of the chlorides by the method of Salkowski-Volhard . . . 390
direct method . . . 394
estimation of the chlorides after incineration (according to Neubauer and Salkowski) . . . 395
the phosphates . . . 395
test for the phosphates in the urine . . . 400
quantitative estimation of the total amount of phosphates . . . 401
separate estimation of the earthy and alkaline phosphates . . . 404
removal of the phosphates from the urine . . . 404
the sulphates . . . 405
test for the sulphates in the urine . . . 408
quantitative estimation of the sulphates . . . 409

Chemistry of the urine—*Continued.* PAGE
quantitative estimation of the total sulphates 409
quantitative estimation of the conjugate sulphates 410
neutral sulphur 411
quantitative estimation 413
urea 414
properties of urea 422
urea nitrate 423
urea oxalate 424
separation of urea from the urine 425
quantitative estimation of urea 426
estimation of nitrogen according to Kjeldahl 435
estimation of nitrogen according to Will-Varrentrapp 437
ammonia 439
quantitative estimation 441
Schlösing's method 441
Folin's method 441
uric acid 442
properties of uric acid 448
tests for uric acid 449
quantitative estimation of uric acid 449
xanthin-bases 455
quantitative estimation 456
hippuric acid 457
properties of hippuric acid 458
quantitative estimation of hippuric acid 459
kreatin and kreatinin 460
properties of kreatin and kreatinin 461
test for kreatinin in the urine 462
quantitative estimation of kreatinin in the urine 462
oxalic acid 466
properties of oxalic acid 469
tests for oxalic acid 469
quantitative estimation of oxalic acid 470
Albumins 472
serum-albumin 472
Patein's or aceto-soluble albumin 485
serum-globulin 485
albumoses (peptones) 486
Bence Jones' albumin 487
peptone 489
hæmoglobin 489
fibrin 490
nucleo-albumin 491
histon and nucleohiston 492
tests for albumin 492
tests for serum-albumin 492
nitric acid test 493
boiling test 496
potassium ferrocyanide test 497
trichloracetic acid test 497

Albumins—*Continued.* PAGE
picric acid test . . . 498
Spiegler's test . . . 498
special test for serum-albumin . . . 498
quantitative estimation of albumin . . . 499
old method of boiling . . . 499
volumetric method of Wassiliew . . . 499
Esbach's method . . . 500
differential density method . . . 500
gravimetric method . . . 501
test for serum-globulin and its quantitative estimation . . . 502
tests for albumoses . . . 502
Salkowski's method . . . 502
Bang's method . . . 503
examination for peptone . . . 504
tests for Bence Jones' albumin . . . 504
tests for (mucin) nucléo-albumin . . . 505
tests for hæmoglobin . . . 506
Heller's test . . . 507
the guaiacum test . . . 507
Donogany's test . . . 507
test for fibrin . . . 508
test for histon . . . 508
Carbohydrates . . . 508
glucose . . . 508
tests for sugar . . . 517
Trommer's test . . . 517
Fehling's test . . . 518
Böttger's test with Nylander's modification . . . 518
fermentation test . . . 519
phenylhydrazin test . . . 520
polarimetric test . . . 521
quantitative estimation of sugar . . . 522
Fehling's method . . . 523
Knapp's method . . . 525
differential density method . . . 526
Einhorn's method . . . 526
Lohnstein's method . . . 527
polarimetric method . . . 528
Bremer's diabetic urine test . . . 530
lactose . . . 531
levulose . . . 531
maltose . . . 531
dextrin . . . 532
laiose . . . 532
pentoses . . . 532
Tollens' orcin test . . . 532
Tollens' phloroglucin test . . . 533
animal gum . . . 533
Glucuronic acid . . . 533

PAGE
Inosit . . . 534
Urinary pigments and chromogens . . . 534
normal pigments . . . 535
urochrome . . . 535
uroerythrin . . . 536
normal chromogens . . . 537
indican . . . 537
tests for indican . . . 540
quantitative estimation . . . 541
urohæmatin . . . 543
uroroseïnogen . . . 544
pathological pigments and chromogens . . . 545
blood-pigments . . . 545
hæmatin . . . 545
urorubrohæmatin and urofuscohæmatin . . . 545
urohæmatoporphyrin . . . 546
biliary pigments . . . 548
Smith's test . . . 550
Huppert's test . . . 550
Gmelin's test (as modified by Rosenbach) . . . 550
Gmelin's test . . . 550
biliary acids . . . 550
cholesterin . . . 551
pathological urobilin . . . 551
melanin and melanogen . . . 554
phenol urines . . . 555
alkapton . . . 555
homogentisinic acid . . . 555
blue urines . . . 559
green urines . . . 559
pigments referable to drugs . . . 559
Ehrlich's diazo-reaction . . . 559
benzaldehyde reaction . . . 564
Conjugate sulphates . . . 566
skatoxyl . . . 566
phenol . . . 566
Salkowski's test . . . 566
quantitative estimation . . . 567
pyrocatechin . . . 567
Acetone . . . 568
tests for acetone . . . 569
Legal's test . . . 569
Lieben's test . . . 570
Reynolds' test . . . 570
Stock's test . . . 570
Denigè's test . . . 570
quantitative estimation . . . 571
Diacetic acid . . . 573
Arnold's test . . . 573
Oxybutyric acid . . . 574
Crotonic acid . . . 576

PAGE
Lactic acid . . . 576
Oxyamygdalic acid . . . 577
Volatile fatty acids . . . 577
Fats . . . 578
chyluria . . . 579
galacturia . . . 579
Ferments . . . 579
Gases . . . 580
hydrothionuria . . . 580
Ptomaïns . . . 581
method of examination for ptomaïns . . . 583
Kryoscopic examination of the urine . . . 585
Sediments . . . 585
Microscopical examination of the urine . . . 585
non-organized sediments . . . 588
sediments occurring in acid urines . . . 588
uric acid . . . 588
amorphous urates . . . 589
calcium oxalate . . . 590
ammonio-magnesium phosphate . . . 591
monocalcium phosphate . . . 592
neutral calcium phosphate . . . 593
basic magnesium phosphate . . . 593
hippuric acid . . . 593
calcium sulphate . . . 593
cystin . . . 594
leucin and tyrosin . . . 595
xanthin . . . 598
soaps of lime and magnesia . . . 598
bilirubin and hæmatoidin . . . 599
fat . . . 599
sediments occurring in alkaline urines . . . 600
basic phosphate of calcium and magnesium . . . 600
ammonium urate . . . 601
magnesium phosphate . . . 601
ammonio-magnesium phosphate . . . 601
calcium carbonate . . . 601
indigo . . . 601
organized constituents of urinary sediments . . . 602
epithelial cells . . . 602
leucocytes . . . 605
red blood-corpuscles . . . 610
tube-casts . . . 612
true casts . . . 614
hyaline casts . . . 614
waxy casts . . . 616
pseudo-casts . . . 618
cylindroids . . . 618
formation of tube-casts . . . 618
clinical significance of tube-casts . . . 619

Microscopical examination of the urine—*Continued.* PAGE
spermatozoa 622
parasites 623
vegetable parasites 623
animal parasites 628
tumor particles 630
foreign bodies 630

CHAPTER VIII.

TRANSUDATES AND EXUDATES.

Transudates 631
general characteristics 631
specific gravity 631
chemistry of transudates 633
microscopical examination 634
Exudates 634
serous exudates 634
hemorrhagic exudates 634
tuberculosis 635
cancer 636
technique 637
bacteriological examination of 637
chemistry of 638
pus 640
general characteristics of pus 640
chemistry of pus 640
microscopical examination of pus 641
leucocytes 641
giant corpuscles 642
detritus 642
red blood-corpuscles 642
pathogenic vegetable parasites 642
protozoa 643
vermes 643
crystals 643
technique 643
gonorrhœal pus 644
the gonococcus 644
putrid exudates 647
chylous and chyloid exudates 647

CHAPTER IX.

THE CEREBROSPINAL FLUID.

Amount 650
Appearance 650
Specific gravity 651

PAGE
Reaction 652
Chemical composition 652
Microscopical examination 653
Bacteriology 654
Toxicity 656

CHAPTER X.

THE EXAMINATION OF CYSTIC CONTENTS.

Cysts of the ovaries and their appendages 657
Hydatid cysts 659
Hydronephrosis 659
Pancreatic cysts 660

CHAPTER XI.

THE SEMEN.

General characteristics 661
Chemistry of the semen 661
Microscopical examination of the semen 662
Pathology of the semen 663
The recognition of semen in stains 664

CHAPTER XII.

VAGINAL DISCHARGES.

Bacteriology 666
Vaginal blennorrhœa 668
Menstruation 668
The lochia 668
Vulvitis and vaginitis 669
Membranous dysmenorrhœa 669
Cancer 669
Gonorrhœa 669
Abortion 670

CHAPTER XIII.

THE SECRETION OF THE MAMMARY GLANDS.

The secretion of milk in the newly born 672
Colostrum 672
The secretion of milk in the adult female 673
Human milk 673
The milk in disease 674
determination of the specific gravity 675
estimation of the fat 677
estimation of the proteids 677

CLINICAL DIAGNOSIS.

CHAPTER I.

THE BLOOD.

GENERAL CONSIDERATIONS.

If blood is allowed to flow directly from an artery into a vessel surrounded by a freezing-mixture, and containing one-seventh its volume of a saturated solution of sodium sulphate, or a 25 per cent. solution of magnesium sulphate (1 volume to 4 volumes of blood), it will be observed that after some time a sediment, presenting the color of arterial blood, has formed at the bottom, which is covered by a layer of clear, straw-colored fluid—the blood-plasma. Upon microscopical examination the sediment will be seen to contain :

a. Numerous homogeneous, non-nucleated, circular, biconcave disks. These measure on an average 7.5 μ in diameter, and are of a faint greenish-yellow color when viewed through the microscope, while *en masse* they present the color of arterial blood—the erythrocytes or red corpuscles of the blood.

b. Roundish or irregularly shaped nucleated cells which are for the most part granular and far less numerous than the red corpuscles, and devoid of coloring-matter—the leucocytes, colorless or white corpuscles of the blood.

c. Minute colorless disks, measuring less than one-half the diameter of a red corpuscle—the so-called blood-plaques, or blood-plates of Bizzozero.

GENERAL CHARACTERISTICS OF THE BLOOD.

Color.

Chemical examination of the blood shows that its color is referable to the presence of an albuminous, iron-containing substance—hæmoglobin—in the bodies of the red corpuscles, which is characterized by its great avidity for oxygen, and forms a compound therewith, known as oxyhæmoglobin. The relatively larger amount of the

latter encountered in the arteries, as compared with the veins, causes the difference in the appearance of arterial and venous blood, the former presenting a bright scarlet-red, the latter a dark-bluish color. A bright cherry-red color is noted in cases of poisoning with carbon monoxide, while a brownish-red or chocolate color is observed in cases of poisoning with potassium chlorate, anilin, hydrocyanic acid, and nitrobenzol. A milky appearance is frequently seen in cases of well-marked leukæmia. In chlorosis and hydræmic conditions, as would be expected, the blood is pale and watery.

Odor.

The peculiar odor of the blood, which varies in different animals, the *halitus sanguinis* of the ancients, is due to the presence of certain volatile fatty acids, and may be rendered more distinct by the addition of concentrated sulphuric acid.

Specific Gravity.

The specific gravity of the blood in healthy adults varies between 1.058 and 1.062, being higher on an average in men, 1.059, than in women, 1.056, and children—boys 1.052, girls 1.050. Generally speaking, it is proportionate to the amount of hæmoglobin and the volume of the red corpuscles. It is diminished to a certain extent by fasting, the ingestion of solids and liquids, gentle exercise, pregnancy, etc. The specific gravity, moreover, depends upon the bloodvessel from which the specimen is taken, being higher, generally speaking, in venous than in arterial blood.

Under pathological conditions the specific gravity may vary between 1.025 and 1.068. In nephritis, chlorosis, the anæmias in general, and in cachectic conditions (carcinoma of the stomach, etc.) it may diminish to 1.031. In pulmonary phthisis the specific gravity is diminished in the third stage (1.040–1.042), and in the first stage (1.049) in the case of those patients in whom the onset has been very gradual. In the second stage normal figures are obtained (1.058–1.060), corresponding to the relatively high percentage of hæmoglobin (90–95 per cent.) which is then noted, and which is referable no doubt to an actual concentration of the blood (Appelbaum). An increased specific gravity is met with in febrile diseases (typhoid fever, 1.057 to 1.063), conditions associated with pronounced cyanosis (emphysema, fatty heart, uncompensated valvular disease, 1.054 to 1.068), and obstructive jaundice, 1.062.

Methods of Determining the Specific Gravity of the Blood.—**Roy's Method.**—A number of test-tubes are filled with a mixture of glycerin and water in different proportions, so that the specific gravity in the different tubes varies between 1.025 and 1.068.

Blood is then drawn from the tip of a finger, or the lobe of the ear, into a capillary tube connected with an ordinary hypodermic syringe, pressure being avoided. A drop of blood is placed in each tube, in which it will sink as long as the specific gravity of the glycerin mixture is lower than that of the blood, while it will remain suspended in a mixture the specific gravity of which is equivalent to its own.

Roy states that it is important for the purpose of comparison to make such examinations in each case at the same hour, as the specific gravity of the blood has been shown to undergo diurnal variations.

Hammerschlag's Method.—A cylinder, measuring about 10 cm. in height, is partly filled with a mixture of chloroform (sp. gr. 1.526) and benzol (sp. gr. 0.889), having a specific gravity of 1.050 to 1.060. Into this solution a drop of blood is allowed to fall directly from the finger, pressure being avoided, and care taken that the drop does not come in contact with the walls of the vessel. The drop, moreover, should not be too large, as otherwise it will separate into droplets, giving rise to inaccurate results. Should the drop sink to the bottom, it is apparent that the specific gravity of the mixture is lower than that of the blood, necessitating the addition of chloroform. This should be added drop by drop while the mixture is thoroughly stirred. If, on the other hand, the drop should tend toward the surface, it is best to add an amount of benzol sufficient to cause the blood to sink to the bottom, and then to bring it to the proper degree of suspension by the subsequent addition of chloroform. As soon as the drop remains suspended the mixture is filtered, and its specific gravity ascertained by means of an accurate areometer registered to the fourth decimal. The figure obtained is the specific gravity of the blood. The chloroform-benzol mixture may be kept indefinitely.

With practice, results sufficiently accurate for clinical purposes may thus be obtained with an expenditure of very little time.

Instead of the chloroform-benzol mixture, one of chloroform and olive oil may be employed, as suggested by van Spanje. It has the advantage of being less volatile than the other. Three parts of chloroform and one of the oil give a mixture with a specific gravity of 1.056.

Schmaltz and Peiper's Method.—Where delicate scales are available the method of Schmaltz and Peiper may be employed, and is certainly the most accurate: a capillary tube, measuring about 12 cm. in length and 1.5 mm. in width, with its ends tapering to a diameter of 0.75 mm., is filled with blood and carefully weighed, when the weight of the blood, divided by the weight of an equivalent volume of distilled water, will indicate the specific gravity.

As the result of numerous investigations it may now be regarded

as an established fact that with the exception of nephritis, circulatory disturbances, leukæmia, and possibly also post-hemorrhagic anæmia and that resulting from inanition, the specific gravity of the blood varies directly with the amount of hæmoglobin and the volume of the red corpuscles. A simple method is thus given by means of which hæmoglobin estimations can usually be made in the absence of more expensive instruments. In the following table the specific gravities, as obtained with Hammerschlag's method, and that of Schmaltz and Peiper, are given, with the corresponding amounts of hæmoglobin; the figures, however, are probably not quite accurate:

Specific gravity according to Hammerschlag.	Hæmoglobin.	Specific gravity according to Schmaltz and Peiper.	Hæmoglobin.
1.033–1.035	25–30 per cent.	1.030	20 per cent.
1.035–1.038	30–35 "	1.035	30 "
1.038–1.040	35–40 "	1.038	35 "
1.040–1.045	40–45 "	1.041	40 "
1.045–1.048	45–55 "	1.0425	45 "
1.048–1.050	55–65 "	1.0455	50 "
1.050–1.053	65–70 "	1.048	55 "
1.053–1.055	70–75 "	1.049	60 "
1.055–1.057	75–85 "	1.051	65 "
1.057–1.060	85–95 "	1.052	70 "
		1.0535	75 "
		1.056	80 "
		1.0575	90 "
		1.059	100 "

LITERATURE.—Schmaltz, Deutsch. Arch. f. klin. Med., vol. xlvii. p. 145; and Deutsch. med. Woch., 1891, No. 16. Stintzing u. Gumprecht, Deutsch. Arch. f. klin. Med., vol. liii. p. 265. Siegl, Prag. med. Woch., 1892, No. 20; and Wien. med. Woch., 1891, No. 33. Hammerschlag, Ibid., 1890, p. 1018; and Zeit. f. klin. Med., 1892, vol. xxii. p. 475. Schmaltz, Deutsch. Arch. f. klin. Med., 1890, vol. xlvii. p. 145; and Deutsch. med. Woch., 1891, vol. xvii. p. 555. Appelbaum, Berl. klin. Woch., 1901, vol. xxxix. p. 7.

Direct Estimation of the Solids of the Blood.—A few drops of blood (0.2 to 0.3 gramme), obtained by means of a fairly deep incision or puncture into the tip of a finger, moderate pressure being made upon the middle phalanx if necessary, are collected in a watch-crystal. This is at once covered with its fellow and weighed. The specimen (open) is then dried at a temperature of from 60° to 70° C. for twenty-four hours, and again weighed, the weight of the solids being thus ascertained.

In healthy adults the following values were obtained by Stintzing and Gumprecht:

	Average.	Maximum.	Minimum.	Average water.
In men	21.6	23.1	19.6	78.4 per cent.
In women	19.8	21.5	18.4	80.2 "

In conditions associated with chronic anæmia the solids, as would be expected, are always much diminished. In leukæmia, on the other hand, owing to the large number of leucocytes present, a relative increase is observed.

Reaction.

The reaction of the blood during life, owing to the presence of disodium phosphate and sodium carbonate, is alkaline, the degree of alkalinity in terms of sodium hydrate under normal conditions corresponding to 182 to 218 mgrms. for every 100 c.c. of blood. v. Jaksch gives 260 to 300 mgrms. as the normal, and Canard 203 to 276 mgrms.

The alkaline reaction of the blood may be demonstrated by repeatedly drawing a strip of red litmus-paper, thoroughly moistened with a concentrated solution of common salt, through the blood, and rapidly washing off the corpuscles with the same solution, when, as a general rule, the alkaline reaction can be clearly made out.

Small plates of plaster of Paris or clay, stained with neutral litmus solution, may be similarly employed, the blood in this case being washed off with water.

Generally, the alkalinity of the blood is lower in women and children than in men, and is, furthermore, influenced by the process of digestion, exercise, etc. At the beginning of digestion, when hydrochloric acid is being freely secreted, the alkalinity of the blood increases; while later on, when both hydrochloric acid and peptones are reabsorbed, the alkalinity in turn diminishes. Higher values are usually found during pregnancy than in the non-pregnant state.

A decrease is observed following violent muscular exercise, such as forced marches by soldiers, owing, in all probability, to an excessive production of acids in the muscles.

Under pathological conditions a diminished alkalinity of the blood is frequently observed. This is particularly marked in cases of severe anæmia (108 to 145 mgrms. of NaOH), and increases as the number of red corpuscles and the amount of hæmoglobin diminish. In chlorosis, however, the diminution in the number of red corpuscles is accompanied by a normal, or but slightly diminished, alkalinity of the blood as a whole. In leukæmia, pernicious anæmia, nephritis when accompanied by uræmia, various hepatic affections, diabetes, carcinoma, the various profound cachexiæ, pseudoleukæmia, poisoning with carbon monoxide and acids, and finally in highly febrile conditions, as in typhoid fever, and toxic processes in general, the alkalinity of the blood is diminished, the lowest value found corresponding to 108 mgrms. of NaOH. A similar decrease follows the prolonged use of acids, while an increase is brought about by the ingestion of alkalies. An increase in the alkalinity of the blood occurs after a cold bath, and it is interesting to note that this is apparently associated with an increase in the bactericidal power of the blood. Possibly the beneficial effect of cold baths in fever may be explained upon this basis. The supposition that in gout

a diminished alkalinity exists in the intervals between the attacks, and that this increases beyond the normal during the attack, has been proved unfounded.

v. Jaksch employs the following method, a modification of that originally devised by Landois: eighteen watch-crystals are prepared, each containing a mixture of a concentrated solution of sodium sulphate and a $\frac{1}{100}$ and a $\frac{1}{1000}$ normal solution of tartaric acid, in varying proportions, so that crystal

No.		c.c.							c.c.				
I.	Shall contain	0.9	of the	$\frac{1}{100}$	norm.	sol.	of the	acid, and	0.1	of the	conc.	Na_2SO_4	sol.
II.	"	"	0.8	"	"	"	"	"	0.2	"	"	"	"
III.	"	"	0.7	"	"	"	"	"	0.3	"	"	"	"
IV.	"	"	0.6	"	"	"	"	"	0.4	"	"	"	"
V.	"	"	0.5	"	"	"	"	"	0.5	"	"	"	"
VI.	"	"	0.4	"	"	"	"	"	0.6	"	"	"	"
VII.	"	"	0.3	"	"	"	"	"	0.7	"	"	"	"
VIII.	"	"	0.2	"	"	"	"	"	0.8	"	"	"	"
IX.	"	"	0.1	"	"	"	"	"	0.9	"	"	"	"
X.	"	"	0.9	"	$\frac{1}{1000}$	"	"	"	0.1	"	"	"	"
XI.	"	"	0.8	"	"	"	"	"	0.2	"	"	"	"

etc., for each c.c. of the mixture.

Blood is taken, preferably from the back, by means of cupping-glasses, and, before it coagulates, 0.1 c.c. is added to each c.c. of the mixture described, when the reaction is determined in each crystal by means of very sensitive litmus-paper. The amount of acid contained in the specimen exhibiting a neutral reaction in terms of NaOH will then indicate the degree of alkalinity of the blood.

As 150 (molecular weight) parts by weight of tartaric acid ($C_4H_6O_6$) combine with 80 (molecular weight) parts by weight of NaOH, or 75 with 40, according to the equation:

$$C_2H_2(OH)_2\begin{matrix}COOH\\COOH\end{matrix} + 2NaOH = C_2H_2(OH)_2\begin{matrix}COONa\\COONa\end{matrix} + 2H_2O,$$

a normal solution would contain 75 grammes of pure tartaric acid to the liter and a $\frac{1}{100}$ and a $\frac{1}{1000}$ normal solution, respectively, 0.75 and 0.075 gramme. As 1000 c.c. of a $\frac{1}{100}$ normal solution would correspond to 0.4 gramme of NaOH, and 1000 c.c. of a $\frac{1}{1000}$ normal solution to 0.04 gramme, 1 c.c. of the $\frac{1}{100}$ normal solution will represent 0.0004, and 1 c.c. of the $\frac{1}{1000}$ normal solution 0.00004 gramme of NaOH.

Supposing, then, that a neutral reaction was obtained in the crystal containing 0.6 c.c. of the $\frac{1}{100}$ normal solution, the alkalinity of the 0.1 c.c. of blood in terms of NaOH would correspond to 0.00024 gramme of NaOH, or 0.24 gramme for 100 c.c. of blood.

As the alkalinity of the blood rapidly diminishes after being drawn, owing, in all probability, to the formation of an acid caused

by decomposition of the hæmoglobin, it is apparent that the experiment must be performed as rapidly as possible, and not more than one minute and a half should elapse between the withdrawal of the blood and the conclusion of the experiment.

Until comparatively recently this method was the only one available for clinical purposes, and the results detailed above were obtained by its aid. It is open to numerous objections, however, and is too complicated for routine work. Of late, a method suggested by Löwy has attracted much attention, and, to judge from the literature, is destined soon to replace the one described. It is both simpler and more accurate. The results, however, differ considerably from those given above, and a careful revision of the work thus far accomplished with the old method will be necessary before definite conclusions can be reached. In healthy adults while fasting the alkalinity of the blood, according to Löwy, corresponds to about 300 to 325 mgrms. of sodium hydrate for every 100 c.c. of blood. Variations amounting to 75 mgrms., plus or minus, are, however, not uncommon, and, according to Strauss, the unavoidable errors may correspond to 30 mgrms. Some of the results obtained in disease are here given:

Carcinoma œsophagi	227–643
Carcinoma ventriculi	256–635
Ulcus ventriculi	302–460
Anadeny of the stomach	354–360
Alcoholic gastritis	343–379
Chronic enteritis	212–272
Phthisis pulmonalis	450–468
Bronchitis	239–343
Neurasthenia	225–426
Arteriosclerosis	208–344
Chronic arthritis	368–465
Erysipelas	498
Typhoid fever	270–640
Pneumonia	263–464
Septicæmia	443
Leukæmia	368–835
Pernicious anæmia	429
Diabetes mellitus	362–457
Chronic interstitial nephritis	310–409
Chronic parenchymatous nephritis	312–490
Cirrhosis of the liver	272–345

A constant diminution of the alkalinity of the blood was noted by Brandenburg in anæmic conditions (202–239 mgrms. of NaOH), while the total amount of the albumins was at the same time diminished. An increase of both factors occurred in catarrhal jaundice; variable results were obtained in two cases of typhoid fever and in one of pyæmia. In uræmia a material decrease was observed which was not associated with a decrease of the total albumins.

According to Orlowsky, the variations in the alkalinity of the blood which have been noted in various diseases and sometimes in

one and the same disease, by various investigators working with the older methods, are referable to the varying isotonicity of the blood and its varying richness in red corpuscles. Working with *blood-plasma* Orlowsky found a marked diminution of the alkalinity in advanced uræmia, in cancerous cachexia, and in severe cases of diabetes, while in other diseases normal values or at most but slight and exceptional variations were observed.

Löwy's Method.—Five c.c. of blood, obtained from one of the superficial veins of the arm (preferably the median cephalic), are allowed to flow into a small flask provided with a long and partially graduated neck, and containing 45 c.c. of a 0.25 per cent. solution of ammonium oxalate. Coagulation is thus prevented and the blood made lake-colored—*i. e.*, the hæmoglobin is dissolved from the stroma of the red corpuscles. The mixture is then titrated with a $\frac{1}{25}$ normal solution of tartaric acid, using lacmoid paper, soaked in a concentrated solution of magnesium sulphate, as an indicator. The lacmoid paper is prepared as follows:

A mixture of 100 grammes of resorcin, 5 grammes of sodium nitrite, and 5 c.c. of distilled water, is heated on an oil-bath to a temperature of 110° C. A violent reaction occurs at this point, and the flame should be removed before it is reached. The substance is then heated to a temperature of 115°–120° C. until all the ammonia which is evolved during the process has been driven off. The residue, which should be of a pure blue color, is dissolved in water and precipitated with hydrochloric acid. On cooling, the coloring-matter is filtered off with the aid of a suction-pump, and washed with a little water. It is then dissolved in absolute alcohol, filtered, and the solution allowed to evaporate in an atmosphere free from ammonia. One gramme of the pigment, which crystallizes in reddish-brown, glistening platelets, is dissolved in 1000 c.c. of 45 per cent. alcohol; in this solution strips of fine Swedish filter-paper are soaked and then allowed to dry.

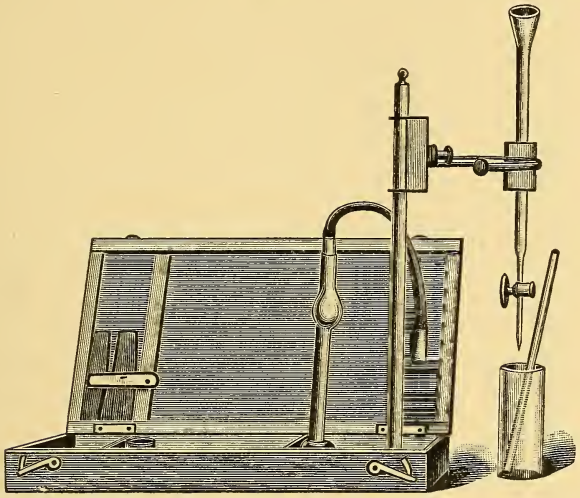
As a normal solution of tartaric acid contains 75 grammes to the liter (see page 22), a $\frac{1}{25}$ normal solution will contain 3 grammes, and 1 c.c. of the $\frac{1}{25}$ normal solution will correspond to 0.0016 gramme of sodium hydrate.

Supposing, then, that 10 c.c. of the $\frac{1}{25}$ normal solution were necessary to neutralize the 5 c.c. of blood, the alkalinity of these 5 c.c. in terms of sodium hydrate would correspond to 0.016 gramme, and the alkalinity of 100 c.c. of blood to $0.016 \times 20 = 0.320$ gramme—*i. e.*, to 320 mgrms.

Engel's Method.—This is essentially a modification of Löwy's method, and is well adapted for clinical purposes, as the amount of blood which is required for a single examination can readily be obtained by ordinary puncture.

The blood is measured and rendered lake-colored in a specially constructed pipette (Fig. 1). To this end, the blood is drawn to the 0.05 c.c. mark and diluted with *neutral* distilled water, so that the volume of the mixture reaches the 5 c.c. line. After slight

FIG. 1.



Engel's alkalimeter.

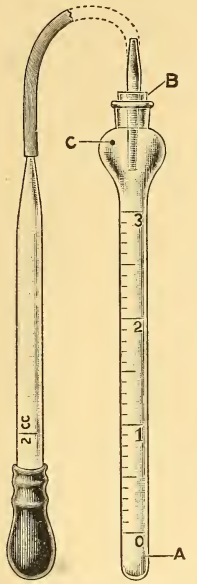
agitation the solution is placed in a small beaker and is titrated with a $\frac{1}{75}$ normal solution of tartaric acid, from a special burette which accompanies the pipette. This is so constructed that each cubic centimeter is divided into twenty parts. Before and after the addition of every drop of the titrating fluid the reaction of the mixture is tested by placing a drop upon Löwy's lacmoid paper (see above). The end-reaction is reached when the yellow drop of the blood mixture shows a distinct red line along the margin. The result is expressed in terms of milligrammes of sodium hydrate per 1 c.c. of blood. Normally about 10 c.c. of the acid solution are employed. The tartaric acid solution contains 1 gramme to the liter, so that 1 c.c. corresponds to 0.533 mgrm. of sodium hydrate.

Supposing that 0.6 c.c. of the acid solution was required to neutralize the 0.05 c.c. of blood, then 12 c.c. would be necessary for 1 c.c. of blood. As 1 c.c. of the acid solution represents 0.533 mgrm. of sodium hydrate, the alkalinity of 1 c.c. of blood would correspond to 12×0.533—*i. e.*, to 6.396 mgrms.

Dare's Method.—This method is based upon the fact that the characteristic spectrum of oxyhæmoglobin disappears at the point of exact neutralization when the blood is titrated with a dilute solution of tartaric acid.

The examination is made with the aid of a special instrument, the *hæmoalkalimeter*, which is pictured in the accompanying illustration (Fig. 2). *B* is a glass stopper through which passes an automatic capillary blood pipette of 20 cbmm. capacity, the exposed end of which is ground to a tapering point. The stopper fits into the tube *A*, which has a capacity of 3 c.c. and is graduated in cubic centimetres. The upper end of the tube is blown into a bulb with a minute aperture at *C*. A 2 c.c. dropping-tube provided with a short piece of rubber tubing accompanies the instrument.

Fig. 2.



Dare's hæmoalkalimeter.

To neutralize the blood, a $\frac{1}{200}$ normal solution of tartaric acid is used, which should contain an amount of alcohol sufficient to prevent the growth of bacteria, but insufficient to precipitate the albumins of the blood. The reagent may be prepared by dissolving 0.075 gramme of tartaric acid (Merck's crystals; guaranteed reagent) in a small amount of distilled water, adding 20 c.c. of alcohol (93–94 per cent.), and diluting to 200 c.c. with water.

For the spectroscopic examination a Browning instrument (Fig. 12) will suffice.

Method.—A drop of blood is obtained from the finger-tip or the lobe of the ear in the usual manner. The blood pipette is filled *in situ* by capillary attraction, holding the instrument horizontally to the drop of blood as it emerges from the wound. With an ordinary medicine-dropper filled with distilled water the blood is washed into the bottom of the tube, connecting the dropper with the pipette by means of a short piece of rubber tubing. Blood and water should just reach the zero mark, and are intimately mixed by closing the aperture in the bulb with the finger and inverting the tube several times. The *reagent pipette* is then filled with the tartaric acid solution and the rubber tubing slipped over the outer end of the blood pipette; by compressing the rubber bulb the acid solution is forced through the pipette into the test-tube, the aperture in the glass bulb being closed before the pressure is relaxed. Having done this the tube is inverted several times while still attached to the reagent pipette, taking care that this is held vertically, so that the acid solution does not get into the rubber bulb. The tube is clamped in front of the spectroscope and examined for the two bands of oxyhæmoglobin (Fig. 4). So long as these are visible more of the acid is added, inverting the tube after each addition; as the bands become fainter one drop at a time is allowed to enter. At first this is rather tedious, but after

several examinations have been made it will be found unnecessary to apply the spectroscope so frequently to determine the point of neutralization, as the eye rapidly learns to recognize this by the characteristic change of color of the blood mixture. The observation is at an end when the oxyhæmoglobin bands have just disappeared.

The examination is made with artificial light, keeping the distance from the light constant.

Dare suggests that for sake of convenience the results be expressed in terms of the number of cubic centimetres of the tartaric acid solution instead of in mgrms. of sodium hydrate, as has been customary. The corresponding values are given in the table below, and have reference to 100 c.c. of blood. His normal values range between 266 and 292.

C.c. of reagent:	Equivalent in terms of mgrms. of NaOH per 100 c.c. of blood.
2.6	345.0
2.4	319.0
2.2	292.0
2.0	266.0
1.8	239.0
1.6	212.0
1.4	176.0
1.2	169.0
1.0	133.0
0.8	96.0
0.6	79.0
0.4	53.0
0.2	26.6

Dare has ascertained with this method that there is a more or less constant relationship between the alkalinity of the blood and the color index, and he suggests that this may be the reason why the results obtained by different investigators differ so widely, as at different stages of the disease the color index may change.

The method is quite convenient and merits the careful attention of all laboratory workers.

LITERATURE.—v. Jaksch, Zeit. f. klin. Med., 1887, vol. xiii. p. 350. A. Löwy, Arch. f. d. gesammte Physiol., 1894, vol. lviii. p. 462. Löwy u. Richter, Deutsch. med. Woch., 1895, vol. xx. p. 526. Peiper, Arch. f. path. Anat., 1889, vol. cxvi. p. 337. Rumpf, Centralbl. f. inn. Med., 1891, vol. xii. p. 447. Kraus, Arch. f. exp. Path. u. Pharmakol., vol. xxvi. Engel, Berlin. klin. Woch., 1898, p. 308. Brandenburg, Zeit. f. klin. Med., vol. xxxvi. p. 267. Orlowsky, Wratch, 1902, vol. xxii. pp. 1190 and 1222. A. Dare, Phila. Med. Jour., Jan. 17, 1903; and Johns Hopkins Hospital Bull., July, 1903.

CHEMICAL EXAMINATION OF THE BLOOD.

General Chemistry of the Blood.

A general idea of the chemical composition of the blood may be formed from the accompanying table of C. Schmidt,[1] calculated for 1000 parts:

[1] Cited by v. Gorup-Besanez, Lehrb. d. physiol. Chem., 4th ed., p. 345.

	Man.	Woman.
Corpuscles	513.00[1]	369.20
Water	349.70	272.60
Hæmoglobin and globulins	159.60	120.10
Mineral salts	3.70	3.55
Plasma	486.90	603.80
Water	439.00	552.00
Fibrin	3.90	1.91
Albumins and extractives	39.90	44.79
Mineral salts	4.14	5.07

If blood is allowed to flow into a vessel and set aside, it will be observed at the expiration of a few minutes that the entire mass has become transformed into a semisolid, gelatinous material, which is spoken of as the blood-clot or the *placenta sanguinis*. Still later it will be seen that a small amount of straw-colored fluid appears on top of the clot, which gradually increases in amount, while the clot itself undergoes shrinkage, until finally it floats, greatly diminished in size, in the surrounding fluid. The straw-colored fluid which has thus been obtained during the process of coagulation is spoken of as the *blood-serum*.

If a bit of the clot is examined microscopically, it will be seen to consist of a more or less dense network of fibres, the meshes of which are filled with blood-corpuscles, which may be washed out, leaving the fibrous network, fibrin, behind.

Chemically speaking, fibrin belongs to the class of the so-called coagulated albumins; it does not occur in the circulating blood, but is formed only during the process of coagulation.

The albumins which are found in the plasma are fibrinogen, serum-globulin, and serum-albumin, but while the last two are likewise encountered in the serum, the fibrinogen has disappeared, and traces of a new albuminous body, fibrino-globulin, are found. There appears to be no doubt that fibrin results from the fibrinogen by a process of dissociation, and that the traces of fibrino-globulin are formed at that time. Modern research, furthermore, has shown that the transformation of fibrinogen into fibrin is dependent upon the action of a special ferment, the fibrin ferment, which is derived in all probability from the leucocytes of the blood by a process of plasmoschisis. The presence of serum-globulin apparently hastens coagulation in an indirect manner, as is done by calcium chloride and the calcium salts in general.

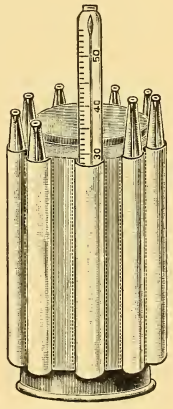
Under normal conditions blood clots in from two to six minutes after being shed, while in disease, notably in hæmophilia, coagulation may be greatly retarded or does not occur at all, so that fatal hemorrhage may follow the infliction of trifling wounds. A tendency to hemorrhage is also observed in scurvy, purpura, in some

[1] This figure is too high; in man it varies between 420 and 470 for 1000 parts of blood.

infectious diseases, such as typhoid fever and yellow fever, in poisoning with phosphorus, etc.[1] Sicard[2] has pointed out that in purpura primary coagulation occurs as with normal blood, but that subsequent retraction of the clot and exudation of serum take place to only a very limited extent. Normal serum when added to fluids, such as hydrocele fluid, which are not spontaneously coagulable, in the proportion of 1 : 80, induce coagulation in from four to six hours. The serum of purpuric patients, on the other hand, is either entirely devoid of this property or possesses it to only a very slight degree. The addition of a trace of calcium chloride, however, causes such serum to behave very much like normal serum. Sicard hence suggests that in certain cases of purpura the fibrin ferment, or its pro-enzyme is not present in sufficient quantity to cause more than a primary coagulation. Subsequent retraction, however, may also be due to the action of another variety of fibrin, the zymogen of which is absent in purpura.

Wright's coagulometer may be conveniently employed to determine the rapidity of coagulation. The instrument is shown in the accompanying illustration (Fig. 3). The essential parts are a tin water can, a thermometer registered to about 50° C., and a set of eight glass tubes measuring about 10 cm. in length with a lumen of 0.25 mm. These tubes are open at both ends and fit into flannel-lined pockets in the leather jacket which surrounds the water can. When the instrument is to be used, the can is filled with water having a temperature about that of the body. The tubes are slipped into their pockets and remain there until they have acquired a similar temperature. They are then successively filled about one-half by aspiration from a drop of blood obtained from the finger or the lobe of the ear at intervals of one minute and replaced (properly numbered) in their pockets. After two and a half to three minutes tube one is examined by attempting to blow its contents upon a sheet of paper; if the blood is still liquid, the second tube is examined, then the third and fourth, etc., until one is found in which the contents have clotted, keeping careful note of the time that has elapsed since each was filled. Under normal conditions the coagulation time with these tubes will be found to vary between three and five minutes. The temperature of the water in the can should be kept uniform during the examination by adding hot water if necessary.

Fig. 3.



Wright's coagulometer.

[1] Schmidt, Pflüger's Archiv, vol. xi. pp. 291 and 515. Bojanus, Inaug. Diss., Dorpat, 1881.

[2] Sicard, Compt. rend. soc. biolog., vol. li. p. 579.

The tubes are cleansed by removing the clots with a fine wire; they are then washed with water, with alcohol, and finally with ether.

Since the formation of fibrin begins as soon as the blood has left its natural channels, it is apparent that absolutely accurate analyses of blood-plasma can hardly be expected. The appended analyses of the plasma of the horse's blood are taken from Hoppe-Seyler and Hammarsten, the figures having reference to 1000 parts:

Water	908.4	917.6
Solids	91.6	82.4
Total albumins	77.6	69.5
Fibrin	10.1	6.5
Globulin	. .	38.4
Serum-albumin	. .	26.4
Fat	1.2	12.9
Extractives	4.0	
Soluble salts	6.4	
Insoluble salts	1.7	

The chief points of difference between plasma and serum are the absence of fibrinogen and the presence of traces of fibrino-globulin, as well as of large quantities of fibrin ferment, in the latter.

From the following table it will be seen that a marked difference exists in the nature of the mineral ingredients between serum and the red corpuscles, the latter being relatively rich in potassium salts and phosphorus, and poor in sodium salts and chlorine. The figures have reference to 1000 parts of blood:

	Man.		Woman.	
	Red corpuscles.	Serum.	Red corpuscles.	Serum.
K_2O	1.586	0.153	1.412	0.200
Na_2O	0.241	1.661	0.648	1.916
CaO	. .	. .	. .	. .
MgO	. .	. .	. .	. .
Fe_2O_5	. .	. .	. .	. .
Cl	0.898	1.722	0.362	1.440
P_2O_5	0.695	0.071	0.643	2.202

It is noteworthy that the amount of sodium chloride in the serum, 6 to 7 pro mille, remains fairly constant no matter whether large amounts are ingested or none at all is given. It is probable that the sodium chloride of the plasma serves the purpose of preventing the hæmoglobin of the corpuscles from being dissolved by the water of the blood. The term "isotonic" has been applied by Hamburger[1] to a salt solution which is just strong enough to prevent the solvent action of the water upon the hæmoglobin of the red

[1] Hamburger, Zeit. f. Biol., vol. xxvi. p. 414; Ibid., vol. xxvii. p. 259; and Virchow's Archiv, vol. cxl. p. 503.

corpuscles. In the case of the serum, however, we meet with a condition of hyperisotonia—*i. e.*, an amount of salt in excess of that actually required in order to prevent the destruction of the red corpuscles, the advantage of which is, of course, apparent, if the variations to which the amount of water in the blood is subject are borne in mind.

In addition to the substances mentioned, the following are also found in the blood:

Fat occurs in amounts varying from 1 to 7 pro mille in fasting animals, while following the ingestion of a meal rich in fats as much as 12.5 pro mille have been encountered.

Soaps, cholesterin, and lecithin have likewise been found.

Sugar, probably glucose, appears to form a normal constituent of the plasma, amounting to from 1 to 1.5 pro mille in man. While it is possible to increase this amount to a certain degree by the ingestion of large quantities of sugar, this appears in the urine, according to Claude Bernard, as soon as 3 pro mille have been exceeded. In addition to glucose, another reducing substance has been found in the blood, which differs from the former in not being fermentable. According to recent researches of P. Mayer,[1] this is in all probability a glucuronic acid compound. Whether jecorin also occurs in the blood is doubtful.

Among the extractives which have been found, there may be mentioned urea, uric acid, kreatin, carbamic acid, sarcolactic acid, glycogen, and hippuric acid, and under pathological conditions xanthin, hypoxanthin, paraxanthin, adenin, guanin, leucin, tyrosin, lactic acid, cellulose, β-oxybutyric acid, acetone, and biliary constituents.

It has been pointed out that the color of the blood is referable to the presence of hæmoglobin in the red corpuscles, and that it varies from a bright scarlet-red in the arteries to a dark bluish-red in the veins, the exact shade depending upon the amount of oxygen present in combination with hæmoglobin as oxyhæmoglobin. Upon chemical examination two other gases may be demonstrated under physiological conditions, viz., carbon dioxide and nitrogen. Of these, the latter appears to play no part in the body-economy, and the amount present merely corresponds to that which would be absorbed by an equal volume of distilled water, viz., 1.8 vol. per cent., calculated at 0° C. and 760 Hgmm. pressure.

The amount of oxygen and carbon dioxide, on the other hand, undergoes considerable variation, depending upon the particular bloodvessel from which the specimen is taken—*i. e.*, whether this be an artery or a vein, and, furthermore, upon the velocity of the blood-current, the temperature of the body, rest, exercise, etc.

The relation existing between the amounts of these gases in arteries and veins may be seen from the following table:

[1] P. Mayer, Zeit. f. physiol. Chem., vol. xxxii. p. 518.

	Arterial blood.	Venous blood.
Oxygen	21.6 per cent.	6.8 per cent.
Carbon dioxide	40.3 "	48.0 "
Nitrogen	1.8 "	1.8 "

Oxygen, as already pointed out, occurs principally in chemical combination with hæmoglobin (oxyhæmoglobin), only 0.26 per cent. being present in solution in the plasma.

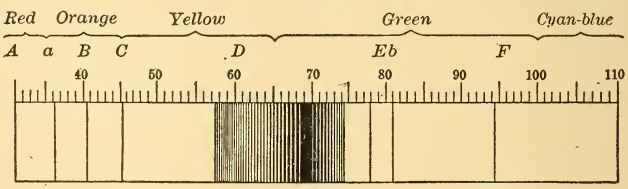
Of the carbon dioxide which may be obtained from the blood, only one-tenth is held in solution, while the remaining portion is found in the red corpuscles, in the form of a loose compound with the alkalies of the corpuscles, and possibly also in combination with hæmoglobin. This portion amounts to about one-third of the total quantity, while the remaining two-thirds are probably held in chemical combination by the alkalies of the plasma and certain albuminous bodies.

The Blood-pigments.

Hæmoglobin and Oxyhæmoglobin.—Hæmoglobin is a proteid in which the albuminous molecule *globin* is combined with the iron-containing pigment *hæmochromogen*. Upon the presence of the latter group depends the great readiness with which hæmoglobin forms compounds with certain gases, such as oxygen, carbon monoxide, carbon dioxide, nitric oxide, and cyanogen. The compound of hæmochromogen and oxygen is termed hæmatin; oxyhæmoglobin is thus the product of globin and hæmatin.

By itself hæmoglobin is largely found in the blood of asphyxia. Under ordinary conditions it is principally present as oxyhæmoglobin; in arterial blood this preponderates, while in venous blood a mixture of both is found.

FIG. 4.



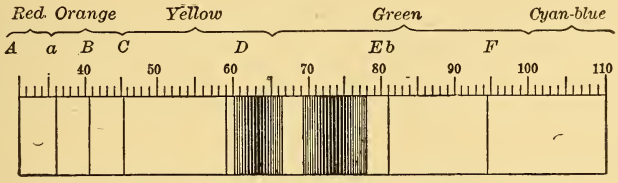
Spectrum of reduced hæmoglobin. (v. JAKSCH.)

On spectroscopic examination hæmoglobin in suitable dilution shows a single band of absorption between D and E, extending slightly beyond D to the left (Fig. 4).

Oxyhæmoglobin shows two bands of absorption between D and E. One band, α, which is not so wide as the second, β, but darker and more sharply defined, borders on D; the second, which is wider but less sharply defined, lies at E (Fig. 5). This spectrum can be

readily transformed into that of hæmoglobin by the addition of a reducing agent, such as an ammoniacal solution of ferrous tartrate (Stokes' fluid), ammonium sulphide, or cuprous salts.

FIG. 5.



Spectrum of oxyhæmoglobin. (V. JAKSCH.)

Under normal conditions the amount of hæmoglobin is fairly constant, but varies somewhat in different countries with the habits of the people, the character of the diet, etc. In Germany, as the result of sixty-one estimations, Leichtenstern found 14.16 per cent. by weight as the average in healthy men, and 13.10 per cent. in women.

Clinically we express the amount of hæmoglobin by relative figures as compared with the average normal percentage by weight; on this basis the scale of the various hæmoglobinometers is constructed. On these instruments the figure 100 represents the average normal value; this, however, varies somewhat with the various forms of hæmoglobinometers according to the average percentage by weight which has been taken as a standard in establishing the 100 mark. With the Gowers instrument (see page 157) Strauss and Rohnstein obtained figures varying between 85 and 125 as normal values; this would furnish an average of 105. Schaumann and v. Willebrandt give 88 as the average normal. With the v. Fleischl instrument (page 153) I rarely find higher values than 90 per cent. in inhabitants of large cities, but with the Dare apparatus (page 151) the average results more nearly approach the 100 mark.

In children the average values are somewhat lower than in the adult. Stierlin gives 79.7 per cent. for boys and 82.1 for girls. Borchmann's values are even lower, viz., 55 and 80; Gundobin gives 70 and 95.

The ingestion of large amounts of water does not cause a dilution of the blood and hence a diminution of the amount of hæmoglobin; but relatively higher values are found upon the withdrawal of liquids, owing to a concentration of the blood as a whole. Fat persons show smaller values than correspond to their age.

A pathological decrease in the amount of hæmoglobin is spoken of as *oligochromæmia*, and is observed in all forms of anæmia from whatever cause.

The lowest values are found in chlorosis, in which the oligochromæ-

mia far exceeds the *oligocythæmia*, viz., the diminution in the number of the red cells (see page 59). In an analysis of 94 cases I found an average of 42.5 per cent.; the lowest value was 17.5 (Fleischl). There are instances on record, however, in which the reading has been even lower.

Very low figures are also seen in splenic anæmia, and it is rare, excepting in chlorosis, to find such a low grade of chromæmia associated with a blood-count which is normal or may indeed be above normal. The average of 13 estimations given by Osler was 47 per cent.

In pernicious anæmia the oligocythæmia exceeds the oligochromæmia. The loss of hæmoglobin is, however, also quite marked, and may be as great as in the most extreme cases of chlorosis. In the series of 23 cases collected by Strauss and Rohnstein the average value was 25 per cent. (Gowers); in 9 cases it was lower than 20 per cent.

In the early stages of leukæmia the loss of hæmoglobin is often not especially marked; later the anæmia may become quite intense, but it is to be noted that the oligochromæmia is not necessarily of high grade even in well-developed cases. Ehrlich thus cites cases in which the Gowers instrument gave readings of from 60 to 70 per cent. On the other hand, there are cases in which the oligochromæmia is an early feature of the disease, and in one instance of this kind I obtained a reading of only 27 per cent.

While in typhoid fever the amount of hæmoglobin is always reduced (Osler), and usually to a greater extent than the number of the red corpuscles, the most severe grades of anæmia may here be encountered during convalescence, when the amount of hæmoglobin may fall to 20 per cent.

In the early stages of carcinoma of the stomach the cachexia is not well pronounced. Schüle states that in his analysis of 198 cases it occurred in only 30 per cent. Later, however, the loss of hæmoglobin is quite marked; the values may indeed approach those seen in chlorosis and pernicious anæmia.

An intense grade of anæmia is produced in cases of generalized septicæmia, and as Ewing remarks no form of the acute disease appears to act more violently than does puerperal or uterine sepsis. A diminution in the amount of hæmoglobin to 20 per cent. is here not uncommon. In the chronic cases also a high grade of oligochromæmia is a constant feature. In a case of lumbar abscess of six months' duration I found 21 per cent. of hæmoglobin, with 1,025,000 red cells. The hæmoglobin in all these cases diminishes more rapidly than the number of the red cells.

In pulmonary tuberculosis a diminution in the amount of hæmoglobin is seen essentially in the third stage of the disease (40–45 per cent.), while previously fairly normal values are obtained (90–95

per cent.). It is to be noted, however, that a certain grade of anæmia (69 per cent.) is quite commonly observed, even in the first stage, in those cases in which the disease has been of very gradual onset, viz., in patients who often have suffered from tubercular affections (scrofula) since childhood. In the third stage the anæmia is well marked (40–50 per cent.) (Appelbaum).

A notable diminution in the amount of hæmoglobin is observed in chronic nephritis, chronic enteritis, in chronic lead and mercurial poisoning, in syphilis, etc.

In syphilis the anæmia develops at a time when the entire organism has been thoroughly infected. The lowest hæmoglobin values are reached just before or coincidently with the appearance of the rash. In the secondary stage the degree of oligochromæmia, *cæteris paribus*, may be regarded as a fair index of the severity of the infection. In untreated cases the hæmoglobin remains low for several days or even for weeks. A gradual rise then occurs which is associated with beginning involution of the exanthem. In uncomplicated cases normal values may subsequently be reached even without treatment; a fall again occurs with relapses. Similar changes are observed in the tertiary stage. Especially interesting are the observations of Justus on the blood-changes which occur in the course of mercurial treatment; Justus ascertained that a rapid and material diminution of the hæmoglobin (10–20 per cent.) occurs when a large (medicinal) amount of mercury is introduced at one time into the body of the infected individual. This decrease is only observed in the blood of patients with florid syphilis; it is specific and does not occur in healthy nor in otherwise diseased individuals. The reaction is demonstrable in every form of syphilitic infection (secondary, tertiary, and hereditary) as soon as the more distant lymph-glands begin to swell. It disappears, or is at least no longer demonstrable with beginning involution of the symptoms.

Justus' Syphilitic Blood-test.—A hæmoglobin estimation should be made on two consecutive evenings at the same hour; on the second evening an inunction is given of not less than 3 grammes of the officinal gray ointment in the case of adults, or of 1 gramme in the case of children. The characteristic drop will then be demonstrable in the course of the following forenoon; examinations should, if necessary, be made at intervals of one or two hours. Justus regards a drop of more than 5 per cent. as evidence of the existence of florid syphilis. His conclusions regarding the diagnostic value of the test are based upon a study of 500 cases. His results have in the main been confirmed, but it is necessary to follow the directions just outlined, as the drop may otherwise be overlooked.

During anæsthesia by ether the amount of hæmoglobin is always absolutely reduced. In some instances there is an apparent increase, but this is never proportionate to the rise in the number of the red

cells which is simultaneously observed (Da Costa, Kalteyer). Owing to the hæmocytolysis which thus undoubtedly takes place a very low percentage of hæmoglobin should be regarded as a counterindication to general anæsthesia. A lower value than 50 per cent. is now regarded by many as a dangerous figure.

For the estimation of hæmoglobin see page 150.

LITERATURE.—Strauss u. Rohnstein, Die Blutzusammensetzung b. d. verschiedenen Anaemien, Hirschwald, Berlin, 1901. Appelbaum, Berl. klin. Woch., 1901, vol. xxxix. p. 7. Quincke, "Zur Pathologie d. Blutes," Deutsch. Arch. f. klin. Med., vols. xxv. and xxvii. Leichtenstern, Unters. über d. Hæmoglobingehalt d. Blutes im gesunden u. kranken Zustande, Leipzig, 1878. W. Osler, "On Splenic Anæmia," Am. Jour. Med. Sci., 1902, vol. cxxiv. p. 763. Justus, Virchow's Archiv, vol. cxl. p. 1; and Deutsch. Arch. f. klin. Med., 1902, vol. lxxv. p. 1.

Hæmoglobinæmia.—The term hæmoglobinæmia has been applied to a condition in which the hæmoglobin is dissolved out from the red corpuscles, and, appearing in the plasma as such, leads at first to a very decided choluria and in extreme cases to hæmoglobinuria.

Various poisons, such as potassium chlorate, carbolic acid, pyrogallic acid, naphtol, arsenic, sulphide of antimony, hydrochloric acid, sulphuric acid, antifebrin, antipyrin, phenacetin, sulphonal, tincture of iodine, when given hypodermically, or even internally in sufficiently large doses, will call forth a hæmoglobinæmia which is followed by hæmoglobinuria.

Fresh morels also contain a poison which is capable of producing an intense hæmoglobinuria, and which may be extracted with hot water.

In acute and chronic infectious diseases of a severe type, such as scarlatina, typhoid fever, intermittent fever, icterus gravis, syphilis, as also in diseases depending upon a hemorrhagic diathesis, such as variola hæmorrhagica, scurvy, as also following insolation, extensive burns, and frostbite, hæmoglobinæmia, leading to hæmoglobinuria, is not infrequently observed. In syphilis a moderate grade of hæmoglobinæmia can be demonstrated by spectroscopic examination of the serum within two or three minutes following an intravenous injection of mercuric chloride in medicinal doses. (See also Justus' test.)

An epidemic hæmoglobinuria of the newly born and a paroxysmal or intermittent hæmoglobinuria, both of unknown origin, have likewise been described.

In a case of Raynaud's disease which I had occasion to observe in the clinic of Dr. H. M. Thomas, at the Johns Hopkins Hospital, hæmoglobinuria at times followed epileptiform seizures.

Hæmoglobinæmia followed by hæmoglobinuria is finally observed after transfusion of the blood of one mammal into the circulation of another.

In some cases, and particularly in those following poisoning with

chlorates, etc., the hæmoglobinæmia ultimately leads to a well-pronounced methæmoglobinæmia (see below).

A hæmoglobinæmia, aside from the urinary examination, may be readily recognized by a spectroscopic examination of the serum, when the two bands of absorption of oxyhæmoglobin will be observed.

A very simple method which may be employed for the same purpose is the following: a small amount of blood is drawn from the patient by means of cupping-glasses and immediately placed on ice, where it is allowed to remain for from twenty to twenty-four hours. At the expiration of this time the clot will have shrunk, floating, if the blood is normal, in the clear, straw-colored serum, while a beautiful ruby-red color is obtained in cases of hæmoglobinæmia. If some of this serum is then heated to a temperature of from 70° to 80° C., the coagulum in the presence of hæmoglobin will present a more or less deep-brown color.

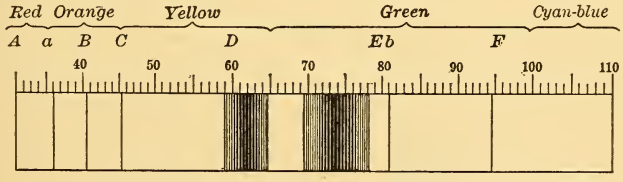
LITERATURE.—Ponfick, Verhandl. d. Cong. f. inn. Med., 1883, vol. ii. p. 205. Stadelmann, Arch. f. exp. Path. u. Pharmakol., 1882, vol. xv. p. 337, and 1884, vol. xvi. pp. 118 and 221. Afanassiew, Zeit. f. klin. Med., 1883, vol. vi. p. 281. v. Jaksch, Verhandl. d. Cong. f. inn. Med., 1891, vol. x. p. 353.

Carbon Monoxide Hæmoglobin.—In cases of coal-gas poisoning the blood, both of arteries and veins, presents a bright cherry-red color, owing to the presence of carbon monoxide hæmoglobin.

Such blood, when properly diluted, like oxyhæmoglobin, shows two bands of absorption between *D* and *E* (Fig. 6), which are nearer the violet end of the spectrum, however, and may readily be distinguished from those referable to oxyhæmoglobin by the addition of a reducing agent. This will not affect the spectrum of carbon monoxide hæmoglobin, while that of oxyhæmoglobin is transformed into the spectrum of reduced hæmoglobin.

For medico-legal purposes a number of additional tests have been devised, among which that suggested by Hoppe-Seyler is one of the simplest and at the same time most reliable. The blood is treated with double its volume of a solution of sodium hydrate (sp. gr.

FIG. 6.



Spectrum of carbon monoxide hæmoglobin. (v. JAKSCH.)

1.3). Normal blood is thus changed into a dirty-brownish mass, which exhibits a trace of green when spread upon a porcelain plate, while carbon monoxide blood yields a beautiful red under the same conditions.

Nitric Oxide Hæmoglobin.—The blood in cases of poisoning with nitric oxide, owing to the presence of nitric oxide hæmoglobin, yields a spectrum which is similar to that of carbon monoxide hæmoglobin; the bands, however, are less sharply defined and paler than those of the latter, and, like these, do not disappear on the addition of a reducing substance.

Hydrogen Sulphide Hæmoglobin (Methæmoglobin Sulphide).—In cases of poisoning with hydrogen sulphide no definite changes can be discovered in the blood upon spectroscopic examination, although Hoppe-Seyler has shown that hæmoglobin may enter into combination with this gas. It is stated, however, that in such cases the blood becomes dark and of a dull-greenish tint, and that the distinction between arterial and venous blood is lost.

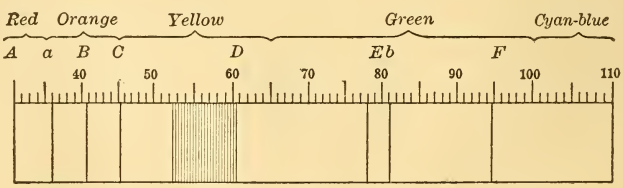
Carbon Dioxide Hæmoglobin.—With carbon dioxide, as mentioned above, hæmoglobin is also thought to enter into combination, the spectrum being similar to that of reduced hæmoglobin. The latter, in fact, is formed artificially when carbon dioxide is passed through a solution of oyxhæmoglobin. If this process is carried further, the hæmoglobin is decomposed and globin is thrown down; an absorption-band is then obtained which is similar to that resulting when hæmoglobin is decomposed with acids (see below), and is no doubt referable to the presence of free hæmochromogen.

Of the blood-changes occurring in cases of poisoning with *hydrocyanic acid* and *acetylene*, but little is known, and the reader is referred to works on toxicology for their consideration.

Hæmatin.—If oxyhæmoglobin in aqueous solution is heated to a temperature of from 60° to 70° C., it is decomposed into globin and hæmatin. The same result is reached by treating the aqueous solution with acids, alkalies, or the salts of various heavy metals.

Hæmatin is an amorphous, blackish-brown or bluish-black substance which is frequently encountered in old transudates, in the stools after hemorrhages, and after meals consisting largely of red meats. It is said to occur in the urine in cases of poisoning with arsenic, and in the blood of animals poisoned with nitrobenzol its presence can likewise be demonstrated with the spectroscope.

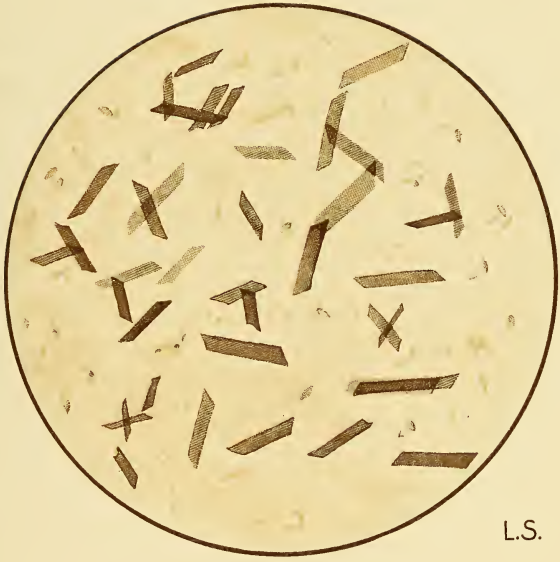
FIG. 7.



Spectrum of hæmatin in alkaline solution. (V. JAKSCH.)

In acid solution it shows a well-defined spectral band between *C* and *D* (Fig. 9). Between *D* and *F* a second band is seen, which

PLATE I.

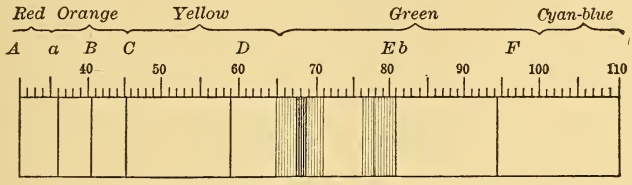


L.S.

Hæmin Crystals.

is much wider but less sharply defined than the first, and may be resolved into two bands by dilution, one between *b* and *F*, near *F*, and another between *D* and *E*, near *E*; a faint fourth band may also be seen between *D* and *E*, near *D*. As a rule only the two bands between *D* and *F* are visible.

Fig. 8.



Spectrum of reduced hæmatin. (v. Jaksch.)

In alkaline solutions it shows but one broad band, the greater portion of which lies between *C* and *D*, extending slightly beyond *D* (Fig. 7).

If an alkaline solution of hæmatin is treated with a reducing substance, reduced hæmatin (hæmochromogen) results, which gives rise to two absorption bands between *D* and *E* (Fig. 8).

Hæmin.—Hæmatin readily combines with one molecule of hydrochloric acid to form hæmin. This substance crystallizes in light- or dark-brown rhombic plates or columns, which are quite characteristic (Plate I.). They bear the name of their discoverer, Teichmann. The size of these crystals varies with the manner in which they are produced, the largest specimens being met with when the glacial acetic acid (see below) is allowed to evaporate as slowly as possible. Specimens measuring from 15 μ to 18 μ in length may then be seen. Smaller crystals will be present at the same time, occurring either singly or in the form of stars, rosettes, and crosses.

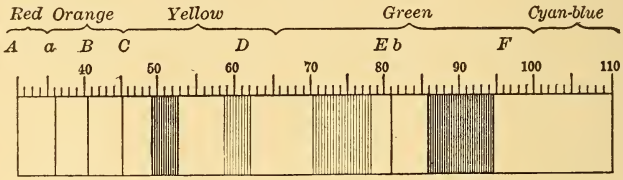
As these crystals may be obtained from mere traces of blood, their formation must be regarded as conclusive evidence in medico-legal examinations. Lewin and Rosenstein have pointed out, however, that under certain conditions a negative result may be reached, even if the coloring-matter is derived from the blood. This is the case especially when the hæmoglobin has been transformed into hæmochromogen or hæmatoporphyrin, or when substances have been mixed with the blood which are either capable of altering its general composition or which, through their mere presence, interfere with the reaction. Such substances are certain salts of iron (rust), lead, mercury, and silver; further, lime, animal charcoal, and sand, when intimately mixed with the blood. In medico-legal cases a spectroscopic examination should hence also be made whenever the hæmin reaction is not obtained.

Method.—A small drop of normal salt solution is carefully

evaporated on a slide, when a few particles of the suspected material, powdered or teased as finely as possible, are placed on the delicate layer of crystallized salt. Glacial acetic acid is now added drop by drop and the specimen carefully heated (three-quarters to one minute) until bubbles begin to form. While evaporation is being continued glacial acetic acid is further added until a light-brown tint appears. As soon as this point is reached, the last traces of the acid are allowed to evaporate, the specimen being held at a greater distance from the flame. A drop of glycerin is then added and the preparation covered with a cover-glass. The examination is made with a one-fifth or a one-sixth objective. Attention is especially directed to brownish streaks or specks, which, in the presence of blood, can usually be made out with the naked eye.

Methæmoglobin.—Methæmoglobin is a pigment closely related to oxyhæmoglobin, and is frequently encountered in hemorrhagic transudates, cystic fluids, and in the urine in cases of hæmaturia and hæmoglobinuria. In the circulating blood methæmoglobin is found after the ingestion of large quantities of potassium chlorate, notably so in children, as also after the inhalation of nitrite of amyl, the use of kairin, thallin, hydrochinon, pyrocatechin, iodine, bromine, turpentine, ether, perosmic acid, permanganate of potassium, and antifebrin (see Hæmoglobinæmia).

Fig. 9.



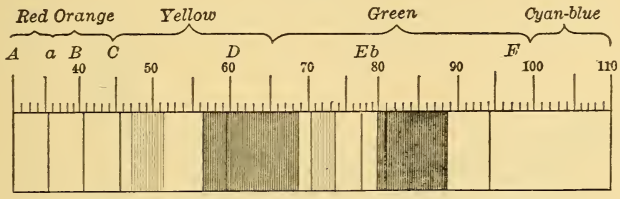
Spectrum of methæmoglobin in acid and neutral solutions. (v. Jaksch.)

The spectrum of an aqueous or slightly acidified solution of methæmoglobin (Fig. 9) closely resembles that of an acid solution of hæmatin, but differs from this in the ease with which it is transformed into that of hæmoglobin when an alkali and a reducing substance are added. The spectrum of hæmatin under the same conditions is transformed into that of an alkaline solution of hæmochromogen. In alkaline solutions, on the other hand, two bands of absorption are observed, which are similar to those of oxyhæmoglobin, but differ from these in the fact that the band nearer *E*, *β*, is more pronounced than the one at *D*, *α*. A third, but very faint, band may further be observed between *C* and *D*, near *D*.

Hæmatoidin.—Small amorphous particles of an orange or ruby-red color, or crystals belonging to the rhombic system, occurring either singly or in groups, are frequently met with in the sputum,

the urine, and the feces, as well as in old extravasations of blood. They were discovered by Virchow, who applied the term hæmatoidin to this particular pigment, the hæmic origin of which is undoubted. It is supposedly identical with bilirubin.

FIG. 10.



Spectrum of hæmatoporphyrin in alkaline solution.

Hæmatoporphyrin.—Hæmatoporphyrin is likewise a derivative of hæmatin, and, according to Nencki and Sieber, isomeric with bilirubin. In dilute solution with sodium carbonate it shows four bands of absorption, one between *C* and *D*, a second one, broader than the first, about *D*, especially marked between *D* and *E*, a third one, not so broad and less sharply defined between *D* and *E*, and a fourth one, broad and dark, between *b* and *F* (Fig. 10).

The clinical significance of this body, which also appears in the urine, as well as the causes which give rise to its formation, are as yet unknown (see Hæmatoporphyrinuria). It has been found post mortem in the blood, in a case of sulphonal poisoning, by Taylor and Sailer.[1]

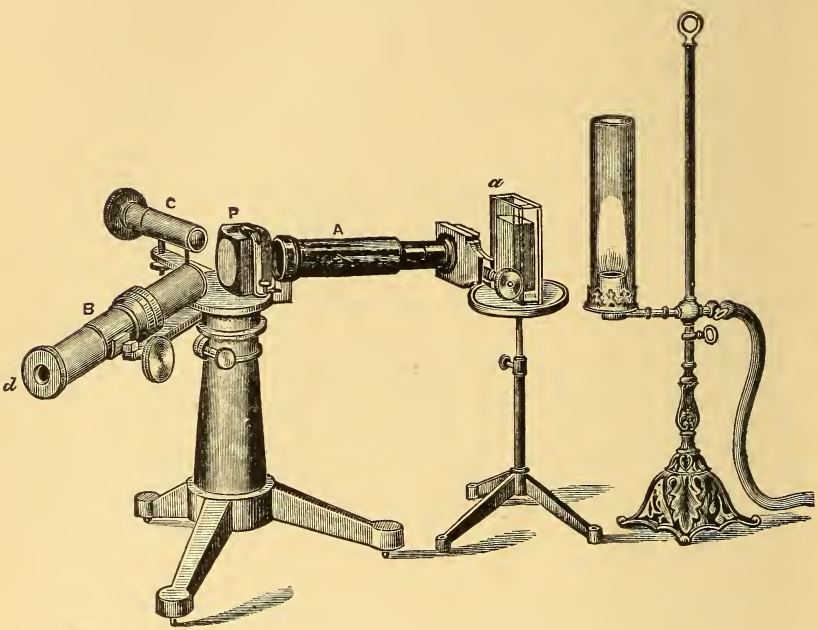
While it is usually possible, as pointed out above, to recognize definitely the presence of blood by the hæmin test, recourse should always be had to a spectroscopic examination whenever the exact nature of the pigment under consideration is to be determined.

The Spectroscope.—The spectroscope (Fig. 11) essentially consists of a tube (A), provided with a slit at its distal end, which may be narrowed or widened, and a collecting-lens at its proximal end. Through the latter, rays of sunlight or of artificial light are thrown upon a prism (P), where they are decomposed into a colored spectrum, which is viewed through an astronomical telescope (B). Through a third tube (C) a fine scale, illuminated by artificial light, is reflected by the prism to the eye of the observer, appearing immediately above the colored spectrum. The left of this is red, passing into yellow, this into green, then into blue, indigo, and finally into violet, which occupies the right end. These colors, however, are not continuous, but are interrupted by a large number of vertically placed dark lines, named after Frauenhofer. The most marked

[1] A. E. Taylor and J. Sailer, Contrib. from the William Pepper Laboratory, Phila., 1900, p. 120.

of these are designated by the letters *A*, *a*, *B*, *C*, *D*, *E*, *b*, *F*, *G*, and *H*. Of these, *A* is found at the left end and *B* in the middle

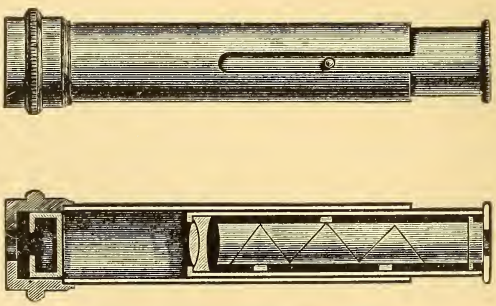
Fig. 11.



The spectroscope. (Neubauer.)

of the red portion of the spectrum, *C* at the boundary of the red and the orange, *D* in the yellow, *E* in the green, *F* in the blue, *G* in the indigo, and *H* in the violet portion; *a* is situated in the red between

Fig. 12.



Browning's spectroscope. (Zeiss.)

A and *B*, nearer *A*, and *b* in the green between *E* and *F*, nearer *E* (see Fig. 4).

If now a colored medium is placed between the slit and the light, not all the rays of colored light reach the eye, but some become ab-

sorbed. In the case of the blood, for example, it may thus be seen that a portion of the yellow and a portion of the red rays are absorbed, a spectrum of this kind being spoken of as an absorption-spectrum.

For clinical purposes various instruments, modifications of the one described, have been devised, among which those of Desego, of Heidelberg, Zeiss, of Jena (Fig. 12), and Hoffman, of Paris, as well as Hering's lensless spectroscope, and Henocque's instrument, are quite serviceable.

THE PROTEIDS OF THE BLOOD.

In considering the proteids of the blood from a clinical point of view, it is necessary to distinguish between an increase and a diminution in their normal amount, constituting the conditions of *hyperalbuminosis* and *hypalbuminosis*, respectively. As may be expected, the former is met with whenever water is more rapidly withdrawn from the system than it can be supplied, and is hence observed in cases of cholera, acute diarrhœa, following the use of purgatives, etc. This increase in the amount of proteids is only a relative increase, however. The occurrence of an absolute increase has not been satisfactorily demonstrated. An absolute hypalbuminosis, on the other hand, is observed following a direct loss of proteids from the blood, as in hemorrhage, dysentery, albuminuria of high degree, the formation of large collections of pus, etc. This is generally associated with a relative increase in the amount of water—*i. e.*, a hydræmia—which is particularly noticeable after hemorrhages, and referable to a diminished secretion and excretion of water, as well as to a direct absorption from the tissues. Hypalbuminosis has also been observed in pernicious anæmia, and is dependent partly upon a diminution in the amount of the albumins of the serum and partly upon a decrease in the weight of the corpuscular solids. The amount of serum-albumin is about normal, while the globulins are much diminished.[1]

The term *hyperinosis* has been applied to a condition in which the amount of fibrin is increased. This is said to occur in various inflammatory diseases, such as pneumonia, pleurisy, acute articular rheumatism, and erysipelas, while a diminished amount of fibrin, *hypinosis*, has been observed in malaria, nephritis, pyæmia, and pernicious anæmia.

In order to determine the amount of fibrin, 30 to 40 c.c. of blood, obtained by means of cupping-glasses, are placed in a previously weighed beaker, fitted with an India-rubber cap, through the centre of which passes a piece of whalebone, firmly fixed. The blood is defibrinated by beating with the whalebone, when the beaker with its contents is weighed, the difference indicating the weight of the

[1] Erben, Zeit. f. klin. Med., 1900, vol. xl. p. 266.

blood. The beaker is then filled with water and the mixture again beaten. The fibrin is allowed to settle and after being washed with normal salt-solution filtered through a filter of known weight. It is further washed with normal salt solution until free from coloring-matter, then boiled in alcohol to dissolve out the fat, cholesterin, and lecithin, dried at 110° to 120° C., and on cooling weighed over sulphuric acid.

In leukæmic blood v. Jaksch[1] was able to demonstrate *peptones* in considerable quantities, and especially so after death, when the amount progressively increased as decomposition advanced. Matthes,[2] on the other hand, could detect no true peptones, but found that the blood contained a deutero-albumose. In one case the serum contained an abundance of nucleo-albumin, derived in all probability from degenerated leucocytes.

More recently albumoses have also been found in a case of abscess of the brain associated with albumosuria. Freund[3] claims that peptones are found in the blood in cases of sarcoma, while in carcinoma they are absent. This statement, however, lacks confirmation.

Following the injection of nuclein and spermin, moreover, albumosæmia appears to occur quite constantly both during the stage of hypo- as well as hyperleucocytosis. After injections of pilocarpin albumosuria is observed only in association with hyperleucocytosis.

In order to test for albumoses, all other proteids should first be removed, when a positive biuret-reaction in the filtrate will indicate their presence (see also Salkowski's test).

Carbohydrates.

Sugar.—Sugar, as indicated above, is a normal constituent of the blood, its quantity varying between 1 and 1.5 pro mille. Under pathological conditions this amount may be exceeded by far, and notably so in diabetes, in which Hoppe-Seyler found as much as 9 pro mille in a given case.

In addition to sugar, a non-fermentable reducing substance has been encountered in the blood, which, according to Mayer's recent investigations, appears to be a compound glucuronate.[4] The presence of jecorin in the blood still remains to be proved.

Large quantities of a reducing substance, the greater portion of which consisted of sugar, have been met with by Trinkler in carcinoma; it was observed at the same time that carcinoma of the internal organs was associated with far greater amounts of sugar than cancerous disease of the skin and the mucous membranes. It is also interesting to note in this connection that an increase in the

[1] v. Jaksch, Zeit. f. physiol. Chem., vol. xvi. p. 243.
[2] Matthes, Berlin. klin. Woch., 1894, Nos. 23 and 24.
[3] Freund u. Obermayer, Zeit. f. physiol. Chem., vol. xv. p. 310.
[4] P. Mayer, Ibid., vol. xxix. p. 59.

degree of the cachexia was not accompanied by an increase in the percentage of sugar.

The results reached by Trinkler[1] apparently also bear out the correctness of the conclusions formed by Freund, who claimed that a differential diagnosis between carcinoma and sarcoma, in which latter condition no increase in the amount of sugar was noted, can always be effected upon the basis of an examination of the blood in this direction.

In the following table the percentages found in the different diseases investigated are given, from which it is apparent that, next to carcinoma, the largest quantities of sugar are met with in the infectious diseases and the lowest figures in diseases of the kidneys:

	Average. Per cent.	Minimum. Per cent.	Maximum. Per cent.
Carcinoma	0.1819	0.1023	0.3030
Typhoid fever	0.0950	0.0875	0.1022
Pneumonia	0.0943	0.0813	0.1092
Dysentery	0.0838	0.0796	0.0915
Heart disease	0.0737	0.0664	0.0897
Peritonitis	0.0701	0.0450	0.0917
Tuberculosis	0.0653	0 0450	0.0817
Syphilis	0.0553	0.0449	0 0748
Nephritis and uræmia	0.0489	0.0321	0.0559

In order to demonstrate sugar in the blood, 15 to 30 grammes, obtained by venesection or cupping-glasses, are placed in an evaporating-dish and treated with an equal weight of finely powdered sodium sulphate and a few drops of acetic acid. The mixture is brought to the boiling-point and passed through a muslin filter as soon as the coagulum has become black and spongy, water having previously been added to the original volume. The filtrate is passed through Swedish paper. In the final filtrate the sugar is then estimated as described elsewhere (see Urine).

Or, the blood is treated with four or five times its volume of alcohol (94 to 96 per cent.) slightly acidified with acetic acid. The mixture is allowed to stand for several hours, no heat being applied. It is then filtered and evaporated on a water-bath until all the alcohol has been driven off. Should any albumin separate out during this process, the residue is again extracted with alcohol. The final residue is dissolved in water. In this solution the sugar is then estimated according to Knapp's method.

Of late, Cavazzani has drawn attention to another method of freeing the blood from proteids, which is said to be entirely satisfactory and less expensive. To this end, 20 to 30 c.c. of blood are added to 200 c.c. of distilled water in a porcelain dish and treated with five or six drops of a solution consisting of 10 parts of acetic acid (sp. gr. 1.040) and 1 part of lactic acid. The mixture is boiled for eight to ten minutes, filtered, and the coagulum washed repeatedly

[1] Trinkler, Centralbl. f. d. med. Wiss., 1890, p. 498. Freund u. Obermayer, loc. cit.

with hot water and finally pressed out in a piece of muslin. The resulting filtrates, which are practically colorless, are then concentrated to a small volume, and any traces of albumin, which may still separate out, filtered off. If an excess of the acid solution has been added, it may happen that the mixture does not clear up on boiling. It is then only necessary to add a few crystals of sodium carbonate, when coagulation will occur at once. On the other hand, it may at times be necessary to add a few more drops of the acetic acid solution.

Williamson's Diabetic Blood Test.—This test is of much interest, and may possibly serve to differentiate the ordinary forms of diabetes from that in which the blood-sugar is not increased. It is based upon the observation that a warm alkaline solution of methylene-blue is decolorized by grape-sugar. As with Bremer's test (see page 63), a positive result may at times be obtained, when the sugar has temporarily disappeared from the urine.[1]

Method.—Twenty cbmm. of blood, obtained from the finger or the ear, are carefully measured off with the aid of the capillary pipette which accompanies Gower's hæmocytometer, and are mixed in a test-tube of small calibre with 40 cbmm. of distilled water. To this mixture 1 c.c. of an aqueous solution of methylene-blue (1 : 6000) and 40 cbmm. of a 6 per cent. aqueous solution of potassium hydrate are added. A control-tube is similarly charged with non-diabetic blood. The two specimens are then placed in boiling water and allowed to remain for from three to four minutes, without shaking. At the end of this time it will be seen that the diabetic blood has decolorized the methylene-blue solution, which has turned a dirty yellowish-green or yellow, while the non-diabetic specimen has retained its original color.

The quantity of blood used should not exceed the amount indicated, as a decolorization of the methylene-blue also results with non-diabetic blood if large amounts, such as 60 cbmm., are employed.

The reaction is supposedly due to an increase of glucose in the blood, and was obtained in all of forty-three cases of diabetes which were examined. It is said to be obtainable for a considerable time after death. Adler[2] found the reaction in all of nine cases of diabetes, while in one hundred and twenty-one non-diabetic cases negative results were reached. Very curiously, it was absent in non-diabetic glucosurias. Adler believes the reaction to be referable to a diminished alkalinity of the blood.

Glycogen.—There appears to be no doubt that glycogen normally occurs in the blood of various animals. Huppert[3] succeeded in

[1] R. T. Williamson, Centralbl. f. inn. Med., vol. xviii. No. 33.
[2] Adler, Zeit. f. Heilk., 1900, vol. xxi. No. 11.
[3] Huppert, Zeit. f. physiol. Chem., 1893, vol. xviii. p. 144.

demonstrating its presence in all animals examined, the amount varying between 0.114 and 1.560 grammes for 100 parts of blood (see also Iodophilia, page 83).

Cellulose.—Cellulose has occasionally been found in the blood of tubercular patients.

Urea.

Urea occurs normally in the blood in traces—0.016 to 0.020 per cent. Larger amounts are encountered whenever, for any reason, as in nephritis, various diseases of the urinary organs, cholera Asiatica, cholera infantum, eclampsia, etc., its elimination is *impeded*, or whenever, as in fever, owing to increased albuminous decomposition, urea is *formed* in abnormally large quantities.

In this connection it is interesting to note that a smaller amount of urea is found in fatal cases of eclampsia than in those ending in recovery, an observation which has been explained by the assumption that in this condition the functional activity, not only of the kidneys, but also of the liver, is lost.

The methods which are available for the detection of urea in the blood are still too complicated for clinical purposes, and the value of the information derived so small as hardly to warrant the labor involved. Hoppe-Seyler's method should be employed whenever an examination in this direction is deemed advisable.[1]

Uræmia.—Formerly, it was thought that the complex of symptoms generally spoken of as uræmia was referable to the retention in the blood of urea or ammonium carbonate. This view has since been disproved, however, although it must be admitted that in uræmia an increased amount of urea is frequently noted. Other views, according to which uræmia is referable to an accumulation of potassium salts, of extractives, and especially of kreatinin, or of ptomaïns in the blood, must still be regarded as being *sub judice*. There is no reason, however, to ascribe the uræmic condition to the retention in the blood of one particular constituent of the urine, and it is not improbable that a retention of all may be responsible for the symptoms observed.

Literature.—Feltz and Ritter, De l'uremie exper., Paris, 1881. Astaschewsky, St. Petersburg. med. Woch., 1881, No. 27. Bouchard, Leçons sur l'autointoxication, Paris, 1887. Rovighi, Rivista clinica, 1886.

Ammonia.

Normal venous blood, according to the researches of Winterberg, contains about 1 mgrm. of ammonia for each 100 c.c. In febrile conditions variable results are obtained, but it appears certain that a definite relation between the height of the fever and the amount of

[1] See Hoppe-Seyler, Handbuch der physiologisch- und pathologisch-chemischen Analyse, Vierte Auflage, p. 363.

ammonia does not exist. In chronic hepatic diseases, and notably in cirrhosis, it is not increased. The course of acute yellow atrophy also is not necessarily associated with an increase. Very significant is the observation that in uræmia following extirpation of the kidneys no increase is observed. An ammoniæmia in the sense of v. Jaksch can hence scarcely be said to exist.

LITERATURE.—Nencki, Pawlow, and Zaleski, Arch. f. exp. Path. u. Pharmakol., 1896, vol. xxxvii. p. 26. Winterberg, Wien. klin. Woch., 1897, p. 330.

Uric Acid and the Xanthin-bases.

Uric Acid.—Formerly, the presence of appreciable amounts of uric acid in the blood was regarded as pathognomonic of gout. But we now know that a lithæmic condition may occur also in other diseases. Traces of uric acid are indeed encountered under normal conditions.

A definite lithæmia has been observed in a variety of disorders, such as pneumonia, acute and chronic nephritis, chronic gastritis, catarrhal angina, conditions associated with an insufficient aëration of the blood, as in the various diseases of the heart, in pleurisy with exudation, emphysema when accompanied by cyanosis, the severer forms of anæmia, etc. v. Jaksch claims to have found uric acid in the blood in 88.88 per cent. of his cases of nephritis. Fever in itself does not appear to lead to an increased production of uric acid, as negative results were obtained in nine cases of typhoid fever out of eleven, in five cases of acute articular rheumatism out of six, etc. The conclusion is thus forced upon us that the diminished alkalinity of the blood observed in nephritis and anæmia is, to some extent at least, dependent upon the presence of a nitrogenous acid, while the diminished alkalinity of the blood observed in fevers is not referable to this cause.

From a survey of the literature upon the subject it appears that an increased elimination of uric acid in the urine is not necessarily accompanied by an increase in the amount of uric acid in the blood. Further researches in this direction are, however, highly desirable, and particularly so in connection with the various forms of gastric disease, in which an increased elimination of uric acid, according to my experience, is so frequently observed.

The assumption that acute attacks of gout are referable to an increased alkalinity of the blood, and a consequent increase in the amount of uric acid, has been disproved.

In order to test for uric acid in the blood, the following method may be employed: 100 to 300 c.c. of blood, obtained by means of cupping-glasses, are at once diluted with three or four times their volume of water and heated on a water-bath. As soon as coagulation sets in, a few drops of a 0.3 to 0.5 per cent. solution of acetic acid are added until a feebly acid reaction is obtained.

After having been kept upon the boiling water-bath for from fifteen to twenty minutes longer, until the albumin has separated out and settled in brownish flakes, the mixture is filtered while hot, and the precipitate washed repeatedly with hot water. Filtrate and washings, which usually present a slightly yellow or brownish color, are again brought to the boiling-point after the addition of 0.3 to 0.5 per cent. of acetic acid, decanted, filtered, and after the addition of a small amount of disodic phosphate further treated according to the Ludwig-Salkowski method (see Urine). The first filtrate is then treated with hydrochloric acid, evaporated to about 10 c.c., and allowed to stand for twenty-four hours, when the uric acid that has separated out is filtered off through asbestos or glass-wool. The filtrate may then be examined for xanthin-bases according to the same method. If no uric acid crystallizes out, as not infrequently occurs, the acid fluid is directly examined for uric acid by means of the murexid test (which see). If upon the addition of ammonia no distinct red color develops, the residue, after thorough desiccation, is dissolved in water, when a reddish color may be regarded as indicating the presence of uric acid, while a yellow or brown color is referable to xanthin-bases. Hopkins' method may also be used.

Garrod's Test.—This test may be advantageously employed if it is desired merely to determine whether or not large amounts of uric acid are present in the blood. A few cubic centimeters of blood-serum (5–10) or of serous fluid, obtained by means of a blister, are placed in a watch-crystal and treated with from six to ten drops of a 30 per cent. solution of acetic acid. A linen thread is immersed in the fluid, which is then kept at a low temperature for from twelve to twenty-four hours. At the expiration of this time a few uric acid crystals will have separated out upon the thread, if the substance is present in large amounts. The true nature of these crystals may then be further determined by the microscope and the murexid test (see Uric Acid in the Urine).

LITERATURE.—Picard, Virchow's Archiv, vol. ii. p. 189. Garrod, Med.-Chir. Trans., 1854, p. 49. Salomon, Zeit. f. physiol. Chem., vol. ii. p. 65; and Charité Annalen, 1880, vol. v. p. 137. Klemperer, Deutsch. med. Woch., 1895, No. 40. Weintrand, Ibid., V. B. p. 185.

Xanthin-bases.—Xanthin-bases do not occur in normal blood or are present only in exceedingly small amounts. Under pathological conditions, however, they may be encountered in recognizable quantities, as in leukæmia, typhoid fever, lymphatic tuberculosis, emphysema, phthisis pulmonalis, pleurisy, and chronic nephritis.

The method above indicated for the demonstration of uric acid in the blood should also be employed when it is found desirable to test for these bodies (see Urine).

LITERATURE.—A. Kossel, Zeit. f. physiol. Chem., 1882, vol. vii. p. 22. Scherer, Verhandl. d. physik. med. Ges. z. Würzburg, 1852, vol. ii. p. 325.

Fat and Fatty Acids.

Quite recently Engelhardt has pointed out that the amount of fat which is contained in normal human blood may be subject to considerable variations, and gives 0.194 per cent. as the average. The lowest figure which he obtained was 0.101 and the highest 0.273 per cent. These figures differ very materially from those of other observers, who have found from 0.73 to 1.4 per cent., but it is quite likely that Engelhardt's method is responsible for these differences, and is probably more reliable (see below). Unfortunately only a few analyses of pathological material have been made with this method, and these have reference only to the blood of cachectic individuals. An increase in the amount of fat has here not been demonstrated, the results varying between 0.112 and 0.284 per cent., with 0.174 as an average. The cachexias in question were of tubercular and carcinomatous origin. With the older methods an increase in the amount of fat, aside from that observed after the ingestion of large amounts of fatty food, has been met with in cases of obesity, chronic alcoholism, in phosphorus-poisoning, in injuries affecting the long bones and the spinal cord, in various hepatic diseases, chronic nephritis, tuberculosis, malaria, cholera, during starvation, pregnancy, in infants at the breast, etc. The greatest increase, however, is observed in certain cases of severe diabetes, in which amounts varying between 1.276 and 18.12 per cent. have been encountered, and in which the fat may be visible with the naked eye (see below).

In such cases fat emboli may be found post mortem, plugging the vessels of various organs, and notably the brain, the lungs, and the kidneys. This increase in the amount of fat constitutes the condition spoken of as *lipæmia*. The term *lipacidæmia* has been applied to the occurrence of volatile fatty acids in the blood, noted by v. Jaksch in various febrile diseases, leukæmia, and at times in diabetes, in which this condition is supposed to stand in a causative relation to the coma. β-oxybutyric acid has been found post mortem in the blood in diabetes.

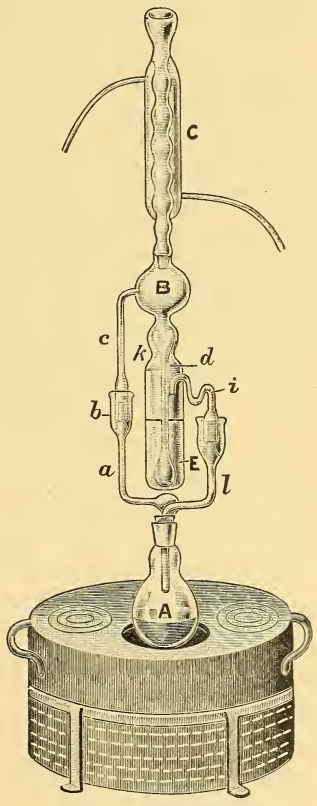
To demonstrate the presence of fat in the blood, it is best to prepare cover-glass specimens, and to mount these in a drop of a 5 per cent. solution of osmic acid. The fat droplets are thus colored black, and appear about as large as the finest fat granules which are found in milk or butter. They may also be stained with Sudan III., and are thus colored red. In every case the necessary instruments and glasses should be carefully cleansed with ether, so as to avoid the accidental introduction of fat.

As a quantitative estimation of the fat is not always possible, Zandy recommends the following simple procedure to demonstrate the presence of an excess of fat: A small drop of blood is received

upon a cover-glass, which is then adjusted over the depression of a cupped slide and ringed with vaselin. On standing, the serum separates out concentrically or excentrically from the small blood-clot, and normally or in the presence of no excess of fat appears perfectly clear. If, however, much fat is present, it becomes cloudy after several minutes or hours, and then appears bluish white, grayish white, or even milky white. To ascertain positively that the turbidity is due to fat, a microscopical examination of the hanging drop is made within a few hours following the preparation of the specimen, so as to exclude fibrin as the possible cause of such turbidity.

Quantitative Estimation.—The apparatus which is best used is a modification of that of Nerking, as suggested by Engelhardt.[1] As seen from Fig. 13, it consists of the ether flask *A*, which is placed on a permanent water-bath, such as that of Münke. *a* represents the escape tube for the ether vapor; at *b* there is a closure by means of mercury, the upper escape tube *c* dipping into the mercury over the mouth of *b*. *B* is the cooler for the ether vapor; *C*, the water condenser. The cooled ether falls through the cooler into *d*. This ends below with a funnel-shaped mouth, close to the bottom of the extraction flask *E*, with five apertures, and has a small open side tube, *f*, which counteracts any negative pressure that may occur above the liquid in the extraction flask. The fluid to be extracted extends to within 1–2 cm. from the aperture of the off-flow tube *i*. When the ether layer extends to the level with *k* the tube *i* acts as a siphon and draws off the fatty ether into *A* again by way of the tube *l*, which is likewise provided with a mercury stop.

Fig. 13.



Fat-extraction apparatus.

The blood, about 10 c.c., is received in a graduate and weighed. It is washed into the extraction flask with about ten times its volume of 2 per cent. hydrochloric acid and boiled for three hours (with inverted condenser). On cooling, the material is extracted in the appa-

[1] The apparatus may be procured from Arno Haak, Jena. Price, 12 marks.

ratus described for about forty-eight hours. At the expiration of this time the fatty ether in *A* is poured into a separating funnel together with the ethereal washings, which are used to remove all the material from the flask, the idea being to get rid of any water or bits of the bloody material that may by chance have been siphoned into *A*. The ether is then evaporated in an open glass dish. The residue is dissolved in absolute ether and filtered through a double folded filter (so as to absorb any traces of water remaining) into a beaker, when the ether is allowed to evaporate. The residue is placed in a drying-oven at 40° C. for one hour, and after remaining in the vacuum over sulphuric acid for twelve hours it is weighed.

With this method lecithins, cholesterins, and fatty acids are obtained conjointly with the fat, which Engelhardt does not regard as objectionable, as they are present only in traces and may be regarded as physiologically equivalent to neutral fat.

To test for fatty acids, 20 to 30 c.c. of blood, obtained by means of cupping-glasses, are treated with an equivalent weight of sodium sulphate and boiled. The filtrate is then evaporated to dryness and extracted with absolute alcohol. Upon evaporation of this solution fatty acid crystals will be obtained, which can readily be recognized with the microscope (see Feces).

LITERATURE.—M. Bönninger, "On the Methods for the Estimation of Fat in the Blood, and the Amount of Fat in Human Blood," Zeit. f. klin. Med., vol. xlii. Parts 1 and 2. T. B. Futcher, "Lipæmia in Diabetes Mellitus," Jour. Am. Med. Assoc., 1899, p. 1006. S. Watjoff, "Ueber d. Fettgehalt d. Blutes b. Nierenkrankheiten," Deutsch. med. Woch., 1897, p. 559. v. Jaksch, "Lipacidæmie," Zeit. f. klin. Med., vol. xi. W. Ebstein, "Beitrag z. Lehre v. d. Lipæmie u. d. Fettembolie," etc., Virchow's Archiv, 1899, vol. clv. p. 571. M. Engelhardt, Deutsch. Arch. f. klin. Med., 1901, vol. lxx. p. 182. Zandy, Ibid., vol. lxx. p. 301.

Lactic Acid.

There appears to be some doubt whether or not lactic acid normally occurs in the blood of man during life. In the blood of dogs, however, Gaglio could always demonstrate the presence of the acid during the process of digestion, after feeding with meat. The amount varied between 0.3 and 0.5 pro mille. During starvation smaller amounts were found, but it never disappeared altogether. In one instance Gaglio obtained 0.17 pro mille after fasting for forty-eight hours. Similar results were obtained by Irisawa, who noted, moreover, that the amount of lactic acid in the blood stood in direct relation to the degree of anæmia which was produced.

In the human being Irisawa found lactic acid fairly constantly after death, the amount, determined as zinc lactate, varying between 0.233 and 6.575 pro mille. These extensive variations he was unable to explain by the character of the disease causing the fatal termination, and it is possible that the cause therefore lies in the fact that in some cases the blood was obtained shortly after death, while in others many hours had elapsed, as Irisawa himself suggests.

The following method may be employed: 100 to 300 c.c. of blood

are extracted with three times its volume of alcohol, filtered, and the filtrate evaporated to a syrupy consistence. This is then made strongly alkaline with barium hydrate and shaken with large quantities of ether, in order to remove the fats which are present. The residue is acidified with phosphoric acid and again shaken with ether for twenty minutes at a time, until the process has been repeated five or six times, the lactic acid passing over into the ether. The ether is distilled off from the extract, the residue taken up with water, and the solution carefully evaporated in order to drive off any ether still remaining, as well as the fatty acids. Carbonate of zinc is now added and the solution heated to 100° C. and filtered. The filtrate is evaporated on a water-bath until crystallization begins, when it is allowed to cool and treated with a few drops of absolute alcohol, in order to effect a complete separation of the lactate of zinc. The solution is allowed to stand exposed to the air until a constant weight is obtained.

LITERATURE.—G. Gaglio, "Die Milchsäure d. Blutes," Du Bois Archiv, 1886, p. 400. T. Irisawa, "Ueber d. Milchsäure im Blut und Harn," Zeit. f. physiol. Chem., 1892, vol. xvii. p. 349.

Biliary Constituents.

Biliary constituents—*i. e.*, bile-pigment and biliary acids—are not encountered in the blood under normal conditions, but are found whenever they are present in the urine (which see). It is noteworthy, furthermore, that bilirubin may frequently be demonstrated in the blood when a urinary examination in this direction yields negative results. According to v. Jaksch,[1] moreover, bilirubin occurs in the blood in nearly every case in which urobilin exists in the urine, which suggests that bile-pigment circulating in the blood may possibly be transformed into urobilin in the kidneys.

A *cholæmia* is encountered in the various pathologic conditions which are associated with a resorption of bile, as in obstructive jaundice, in association with an excessive elimination of bile into the intestinal canal, as well as with an increased destruction of red corpuscles.

In order to test for biliary acids, the blood is first treated with alcohol, in order to remove the proteids. The biliary acids which are present in the filtrate are next transformed into their lead salts by means of lead acetate and ammonia, and thus precipitated. After washing with water the precipitate is boiled with alcohol and filtered. The lead salts are decomposed by means of sodium carbonate, the solution is again filtered, the filtrate evaporated to dryness, and the residue extracted with absolute alcohol. The alcohol is distilled off, when the biliary salts of sodium will crystallize out or remain behind as an amorphous mass, which may be tested directly

[1] v. Jaksch, Clinical Diagnosis, 4th ed., 1896, p. 97.

according to Pettenkofer's method. To this end, some of the residue is dissolved in water and treated with two-thirds of its volume of concentrated sulphuric acid, care being taken that the temperature does not rise beyond 60° C. To this mixture a few drops of a 20 per cent. solution of cane-sugar are added, when in the presence of biliary acids a beautiful violet color is obtained, which is referable to the action of furfurol, formed from the cane-sugar and the acid, upon the biliary acids.

Bilirubin can be demonstrated in the blood most readily in the following manner: 10 to 15 c.c. of blood obtained by means of cupping-glasses, are allowed to coagulate, when the serum is removed by means of a pipette, filtered through asbestos, and coagulated in as thin a layer as possible at a temperature of 80° C. Under such conditions normal serum presents a light straw color, while in the presence of biliary coloring-matter a light greenish color is seen, which becomes grass green on standing. Should the serum contain hæmoglobin, as in hæmoglobinæmia, a brownish color results.

Acetone.

Acetone has been found in the blood in considerable amounts under various pathological conditions, and especially in fevers.

In order to demonstrate its presence, the blood is first extracted with ether and subsequently distilled, when the distillate is tested as indicated elsewhere (see Acetonuria).

Denniges's test may also be employed, and has the advantage of greater simplicity: 3 c.c. of blood are treated with about 30 c.c. of Denniges's reagent and allowed to stand until the dark-brown precipitate has settled to the bottom. The supernatant fluid is filtered off and treated with a little more of the reagent, so as to insure *complete* precipitation. It is then acidified with sulphuric acid and heated as described. The formation of a white precipitate, which is soluble in an excess of hydrochloric acid, is referable to acetone or diacetic acid.

Literature.—v. Jaksch, Acetonurie u. Diaceturie, Berlin, 1885. Reale, Schmidt's Jahrbüch., 1892, p. 106 (Extract).

MICROSCOPICAL EXAMINATION OF THE BLOOD.

The Red Corpuscles.

Variations in Size and Form.—The normal red blood-corpuscles are greenish-yellow, circular, biconcave disks, which in post-embryonic life are non-nucleated. Their diameter varies between 6 and 9 μ, with an average of 7.5 μ. The presence of larger or smaller cells is abnormal. Smaller cells are termed *microcytes*, and measure from 3.5 to 6 μ; larger cells are known as *macrocytes* or *megalocytes*, and

usually have a diameter of from 9.5 to 12 μ; still larger specimens are spoken of as giant corpuscles (Hayem);[1] they may attain a diameter of 16 μ. The terms *microcytosis* or *microcythæmia* and *macrocytosis* or *macrocythæmia* are used to designate a predominance of the corresponding variety.

As regards the origin of the macrocytes, there is evidence to show that they may result from the common normocytes in the circulating blood through increased imbibition of plasma, so that their occurrence from this point of view could be regarded as a degenerative phenomenon. But, on the other hand, the presence of macrocytes may be interpreted as evidence of a regenerative process, bearing in mind that in the bone-marrow the size of the erythroblasts is larger than that of the common normocytes; the macrocytes would thus represent young normocytes which have prematurely found their way into the circulation. The microcytes probably result from the normocytes in the circulating blood through loss of plasma; whether their presence may at any time be regarded as the expression of a regenerative process seems questionable. Not infrequently microcytes are formed artificially during the preparation of the specimen.

Microcytosis is, on the whole, of comparatively little clinical interest, and may be observed in any severe anæmia. Macrocytosis is more important. To a certain extent it is seen in severe forms of anæmia of whatever origin, but it is noteworthy that the presence of macrocytes in large numbers is essentially observed in pernicious anæmia. During the active period of the disease the macrocytes may here represent 70 per cent. of all red cells (Lazarus). The condition, however, is not constant.

Going hand in hand with pathological variations in the size of the red corpuscles, there are variations in form which may affect not only the microcytes and macrocytes, but also the corpuscles of normal size. Cells may thus be seen which resemble a flask, a kidney, a biscuit, a boat, a balloon, a dumb-bell, or an anvil, while others are altogether irregular in appearance (Plate II., Fig. 2). Especially interesting is the fact that such abnormally formed cells, which are generally spoken of as *poikilocytes*, may manifest a certain degree of motility, so that they have at times been mistaken for microparasites. This is seen especially in marked cases of pernicious anæmia, and is most noticeable in the smaller forms. In pernicious anæmia *poikilocytosis* is most pronounced, and at one time it was thought that the condition was characteristic of the disease. It has been shown, however, that it occurs in other anæmias as well, though its occurrence is probably always evidence of a specially severe form. In chlorosis it is usually only seen in the most severe cases, and particularly in those manifesting a tendency to thrombosis and embolism.

In this connection a special deviation from the normal form of the

[1] Hayem, Le Sang, Paris, 1891.

red corpuscles also requires consideration, viz., the prevalence of oval cells. These are notably observed in pernicious anæmia and seem to be of distinct diagnostic importance. They are found not only during the active periods of the disease, but frequently also in the interval between exacerbations.

Poikilocytosis is a degenerative phenomenon, and it is essential not to mistake for the pathological variety certain abnormalities in form which may be seen in any normal preparation. Such deviations from the circular form are the result of mechanical injury, mutual compression, etc., and can readily be distinguished with a little practice.

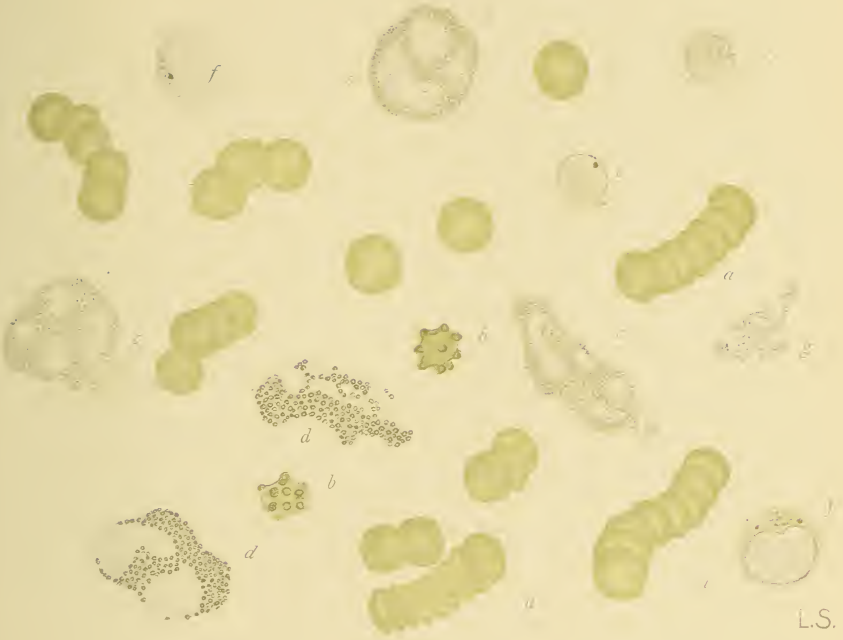
In wet preparations red cells will be seen near the margin of the drop where evaporation is actively going on, which present little knobs or spicules on their surface and along the periphery. Such cells are spoken of as crenated cells. The phenomenon in itself is normal, but it is noteworthy that *crenation* may at times be observed in the centre of a carefully prepared specimen after a few seconds, while as a rule from fifteen to thirty minutes elapse before the process of crenation begins to attack cells in this location. The significance of this early crenation is not known. This is also true of delayed *money-roll formation*, which is at times observed in various hepatic diseases, in pneumonia, nephritis, etc., whereas normally the red corpuscles tend to agglutinate in this form immediately unless special pains have been taken to secure the separation of the individual cells (Plate II., Fig. 1).

Variations in the Color of the Red Corpuscles.—The degree of coloring of the red corpuscles depends upon the amount of hæmoglobin. Owing to the biconcavity of the cells the centre in well-mounted specimens is always paler than the periphery, and any deficiency in the amount of coloring-matter is here at once apparent. With a moderate grade of anæmia the cell as a whole looks paler, and the pale central area is increased in size. With a further increase in the loss of coloring-matter the central area is absolutely colorless and encroaches upon the peripheral colored zone more and more until finally the so-called *pessary forms* result, in which only a narrow rim of hæmoglobin remains. These changes can be made out in wet preparations, but are especially well seen in stained specimens. The central pale area is, however, visible only in well-preserved cells; in every spread many corpuscles will be seen which appear merely as flattened disks, and are uniformly colored throughout; the biconcave structure has here been lost as the result of mechanical injury.

The color of the normal red cells in wet specimens is a pale greenish yellow. In malaria curiously discolored corpuscles are frequently seen, which present a bronzed appearance; their presence should always excite suspicion. The meaning of the discoloration is

PLATE II.

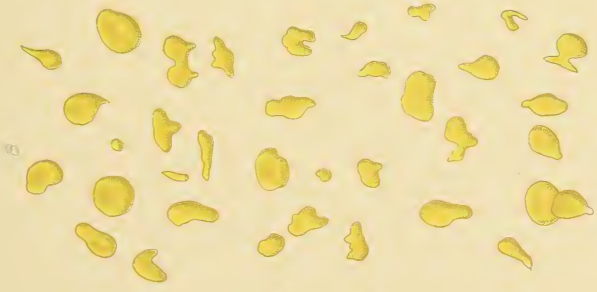
FIG. 1.



The Elements of Normal Blood.

a, red cells in rouleaux; *b*, crenated red cells; *c*, finely granular (neutrophilic) leucocytes; *d* coarsely granular (eosinophilic) leucocytes; *e*, small and *f*, large mononuclear leucocytes; *g*, plaques.

FIG. 2.



Poikilocytosis.

Unstained specimen taken from a case of pernicious anæmia.

not known, but in all probability it is evidence of a degenerative process.

The Color Index.—The term color index is used to designate the relative amount of hæmoglobin which is contained in each corpuscle. It is determined by dividing the percentage of blood coloring-matter by the percentage of red cells as compared with the recognized normal, viz., 5,000,000.

EXAMPLE.—The percentage of hæmoglobin is 50, the red count per cbmm. is 2,000,000, viz., 40 per cent. of the recognized normal, 5,000,000. The color index is then 50 divided by 40—*i. e.*, 1.25.

Under normal conditions the color index is about 1, but may vary from 0.95 to 1.17; it is slightly higher in men than in women. In the secondary anæmias, in which the decrease in the amount of hæmoglobin is proportionate to the diminution of the red corpuscles, the color index is approximately normal. But in the majority of cases the diminution of the hæmoglobin somewhat exceeds that of the red cells, so that lower values are commonly met with. In pernicious anæmia, on the other hand, where the corpuscular decrease usually exceeds the diminution of the hæmoglobin, a high color index is the rule. There may be periods in the course of the disease, however, in which a normal index and even subnormal values are found. In the chronic cases lower figures are more commonly obtained than in the acute cases. In the series of 22 cases collected by Strauss and Rohnstein the value of the color index varied between 0.5 and 1.95. In 8 cases of the series variations from 1.13 to 1.95 were observed, and in 6 lower values than 1 were noted, viz., 0.5–0.9. Cases in which the color index falls as low as 0.5 are rare in pernicious anæmia. In the one instance in the series in which this was found, the hæmoglobin was only 10 per cent., while the red cells numbered 1,048,000; there was a high grade of poikilocytosis and all transitions between the smallest microcytes and the largest types of macrocytes.

In the secondary anæmia of carcinomatosis the color index rarely exceeds 1. In Strauss and Rohnstein's series[1] of 35 cases the highest value was 1.1 (in one case only); in the rest it varied between 0.53 and 0.96.

In chlorosis, in which the degree of corpuscular diminution usually exceeds that of the hæmoglobin, the color index is markedly lower; in especially severe cases it may fall to 0.3 and even lower. But it is not admissible to make the diagnosis of chlorosis on this basis only, as it is fairly common to meet with a markedly lowered color index in some secondary anæmias also, and especially in the form which is referable to carcinoma. In splenic anæmia likewise the degree of oligochromæmia may far exceed the degree of oligocythæmia.

[1] Strauss u. Rohnstein, Die Blutzusammensetzung b. d. verschiedenen Anaemien, Berlin, 1891.

Variations in Number.—The number of red corpuscles in the blood of healthy adults is fairly constant. In man 5,000,000 may be considered a fair average, and in women 4,500,000. Higher values are not uncommon, but rarely exceed 6,000,000 in perfectly normal individuals.

The highest figures are normally obtained at birth. In a study of seventeen infants Hayem found 6,260,000 as the highest and 4,340,000 as the lowest count. Soon after birth the number diminishes, but later it rises again, until the normal adult values become established about the time of puberty.

A somewhat higher average is found among people living at a considerable elevation above the sea level, and it is interesting to note that an increase in the number occurs whenever a change in the habitation is made from a lower to a higher level. This increase is frequently quite marked, as is apparent from the following table, which is taken from Ehrlich :[1]

Altitude	Increase of
561 metres	800,000
700 "	1,000,000
1800 "	2,000,000
4392 "	3,000,000

A corresponding diminution occurs when a change is made from a higher to a lower level.

In this connection Gaule's[2] observations are especially interesting. On the occasion of a balloon ascension to a height of from 4200 to 4700 metres he counted 7,040,000, 8,800,000, and 7,480,000, respectively, in the three participants of the journey. The hæmoglobin was at the same time diminished, and he accordingly concluded that the increase during the ascent was due to an increased production of red cells ; the probable nature of this conclusion was further strengthened by the fact that numerous normoblasts were found in the blood, many undergoing division. Jolly and Bensaude[3] on similar expeditions were unable, however, to demonstrate the presence of nucleated red cells. According to Weinzirl,[4] the increased counts due to high altitude are temporary and in part at least referable to cold. He showed that in rabbits a certain increase in the number of red cells occurs when they are removed from warm to cold quarters, and that their subsequent removal to a higher altitude does not lead to a further increase.

Clinically we distinguish between *relative polycythæmia* in which the condition is due to a diminution in the quantity of the plasma,

[1] P. Ehrlich u. A. Lazarus, " Die Anæmie," Nothnagel's specielle Path. u. Therap., vol. viii. part 1.

[2] Gaule, Compt. rend., vol. cxxxiii. p. 903.

[3] Bensaude, Compt. rend. Soc. biol., vol. liii. p. 1084.

[4] Weinzirl, Am. Jour. Med. Sci., 1903, vol. cxxvi. p. 299.

and *true polycythæmia* in which there is an actual increase in the number of the red corpuscles. Relative polycythæmia is much the more common. It may be referable to loss of liquid, either by sweating, diarrhœa (by far the most common), or increased diuresis. In another group of cases there is loss of liquid by secretion or transudation, as in narrowing of the pylorus with dilatation of the stomach, and in the constant loss of liquid from the blood in recurring ascites. In some of these cases the polycythæmia is of high grade, and may persist for years. The polycythæmia which is noted in poisoning by phosphorus and carbon monoxide, during and immediately after the administration of ether, following cold baths and severe muscular exercise, probably also belongs to this order and is no doubt referable to vasomotor disturbances. Of similar origin no doubt is the polycythæmia which is noted in disease of the adrenal glands, where counts of from 6,000,000 to 7,000,000 have been repeatedly noted; and the same is probably true of diabetes, in which polycythæmia may be observed both while fasting and while much fluid is being ingested.

True polycythæmia is met with in diseases in which there is difficulty in proper aëration of the blood, as in heart disease,[1] and in a peculiar type of chronic cyanosis which has recently been described by Osler[2] as a new clinical entity. In acquired heart disease with continued inadequacy of the circulation of slight degree a moderate grade of polycythæmia is very common; in the congenital form the figures often reach 8,000,000 to 9,000,000. The highest values are seen in Osler's disease. In the nine cases which have been thus far reported the highest count was 12,000,000; in eight of the cases it was above 9,000,000, and in the ninth it was 8,250,000. The usual range of hæmoglobin at the same time was from 120 to 150; the specific gravity varied between 1.067 and 1.083, and the leucocyte count between 4000 and 20,000; as a rule it was below 10,000.

While there can thus be no doubt that a true polycythæmia does occur, it has been conclusively demonstrated that such a condition does not exist in what is generally termed *true plethora*, and that the various symptoms of plethora formerly attributed to a general increase in the amount of blood are more likely referable to vasomotor disturbances.

Oligocythæmia, viz., a diminished number of red cells, is much more common than polycythæmia. It may be temporary or permanent, and is seen in all forms of anæmia of whatever origin. It is most marked in pernicious anæmia. The exact figure will here, of course, depend upon the stage of the disease and the individual case. A decrease to one-half of the normal number may be seen in com-

[1] Stengel, Proc. Path. Soc. Phila., 1899, Oertel, Deutsch. Arch., vol. l. p. 293.
[2] W. Osler, "Chronic Cyanosis with Polycythæmia," Am. Jour. Med. Sci., 1903, vol. cxxvi. p. 187.

paratively mild cases; a million red cells is a common count. The number may fall to 500,000 and even lower. In one case reported by Quincke[1] a count of 143,000 was observed, and it is interesting to note that seventy-four days later the same patient had 1,234,000 per cbmm. Osler[2] reports a case in which shortly before death the red cells fell below 100,000. This is the lowest count that has been recorded. In the stage of amelioration they may rise to 4,000,000 and even higher. In the series collected by Strauss and Rohnstein[3] 1,240,000 was the average at the time when the patient first came under observation, and in Cabot's series of one hundred and ten cases the average number is almost identical—1,200,000.

In chlorosis, contrary to what is found in pernicious anæmia, the red cells are usually not much diminished. In Cabot's[4] series of seventy-seven cases the average count was 4,050,000. At times, however, cases are met with in which the diminution of the red cells almost keeps step with the diminution in the amount of hæmoglobin. v. Limbeck cites three cases with 1,750,000, 1,850,000, and 1,930,000, respectively; and Hayem mentions an instance in which only 937,360 cells were counted. Such cases are exceptional.

As in chlorosis so also in splenic anæmia, the corpuscular anæmia is of very moderate grade, even though the diminution in the amount of hæmoglobin may be considerable. Of the forth-one cases collected by Osler, the average was 3,425,000; the lowest count was 2,187,000 and the highest 5,200,000.

In leukæmia the red cells are usually not diminished to a very great extent; and the oligocythæmia is generally more marked in the lymphatic than in the myelogenous variety; the average figures in Cabot's series are 2,730,000 and 3,120,000, respectively. Counts of 1,000,000 or thereabout may, however, be met with.

In pseudoleukæmia the red cells may be only moderately diminished, viz., between 3,000,000 and 4,000,000, but in some cases the corpuscular destruction is quite active, and in the last stages of the disease values may be found which are not much above 1,000,000 or 1,500,000.

An extreme and rapidly progressive anæmia is frequently noted in acute streptococcus infections. Grawitz[5] states that according to Rocher's investigations it is probable that the diminution of the red cells in septicæmia is greater than in any other infectious disease and appears in a shorter time. Cases may indeed be encountered in which the question of pernicious anæmia may enter into the diagnosis, as occurred in two cases of gonorrhœal endocarditis which were observed by Osler.

[1] Quincke, Centralbl. f. d. med. Wiss., 1877, No. 47; and Deutsch. Arch., 1877, vol. xx.
[2] Osler, Johns Hopkins Hosp. Bull., 1902, vol. xiii. p. 251.
[3] Strauss u. Rohnstein, loc. cit.
[4] Cabot, Clinical Examination of the Blood, Wm. Wood & Co.
[5] Grawitz, Klin. pathol. d. Blutes, Enslin, Berlin, 1902.

An extreme grade of corpuscular destruction is also noted in malaria. In acute cases the loss of red cells during the first twenty-four hours may reach 1,000,000, and in two days even 2,000,000. In neglected chronic cases the count usually varies between 3,000,000 and 4,000,000; the oligocythæmia may, however, be far more extensive, and Ewing[1] cites a case observed by Kelsch, with only 583,000 red cells per cbmm.

The anæmia observed after typhoid fever is as a rule not very severe, but exceptional cases occur in which the loss of red corpuscles is considerable. Osler cites an instance in which the number fell to 1,300,000.

The post-rheumatic anæmia is usually not so pronounced.

In acute endocarditis Stengel[2] has noted a rapid decrease of the red corpuscles, often to 50 and even 40 per cent.

In pulmonary tuberculosis the number of the red corpuscles runs a course parallel to that of the hæmoglobin (which see). Oligocythæmia is really only seen during the third stage (2,000,000–2,500,000); while during the second stage, owing to an actual concentration of the blood (Grawitz), normal figures are the rule. In the first stage a diminution of their number (3,800,000) is only seen in patients who have repeatedly suffered from tuberculous affections (scrofula) since childhood, and in whom the onset of the pulmonary disease has been gradual, while, on the other hand, normal values are found in individuals who appear to be in perfect physical health, who are well-nourished, with well-shaped chests, and without hereditary predisposition (Appelbaum).[3]

In acute gastritis, and usually in chronic gastritis also, the number of the red corpuscles is not diminished, while in carcinoma a marked oligocythæmia occurs at some time in the course of the disease. In the earlier stages, however, this is often but little marked, and at times even an apparent increase of the red cells is noted. Later a diminution is probably always found. In the severer forms of chronic gastritis a diminution is also fairly constant, but rarely so marked as in carcinoma, if we except those cases of gastric anadeny which present the clinical picture of a pernicious anæmia. In the differential diagnosis between carcinoma of the stomach and pernicious anæmia a count below 1,000,000 points to the latter disease. In ulcer of the stomach normal values are found unless hæmatemesis has recently occurred or unless the disease is associated with profound chlorosis.

The count which is obtained in post-hemorrhagic cases will depend very largely upon the amount of blood lost and the time at which the examination is made. The lowest counts, according to Lyon,[4]

[1] Ewing, On the Blood, Lea Bros. & Co., 1901.
[2] Stengel, loc. cit., p. 59.
[3] Appelbaum, Berl. klin. med. Woch., 1901, vol. xxxix. p. 7.
[4] Lyon, Virchow's Archiv, 1881, vol. xciv.

Hühnerfauth[1], and Siegel-Maydl,[2] are found between the second and the eleventh day. In Rieder's[3] cases the figures varied between 1,300,000 and 3,335,000; in those of Strauss and Rohnstein,[4] between 1,119,000 and 4,420,000. A sudden reduction in the number to 1,000,000 or less is usually followed by a fatal result.

In the anæmias of infancy and early childhood the oligocythæmia is often very pronounced. In the infantile pseudoleukæmia of v. Jaksch especially low values may be found associated with an increase of the leucocytes of such extent that the ratio between the two may be suggestive of true leukæmia; there is, however, no myelæmia, but an increase of the normal types. In infantile leukæmia of the lymphatic variety McCrae found 2,350,000 as the highest count.

In the majority of cases of rickets there is no material diminution in the number of the red cells, while the hæmoglobin may be much reduced, but in the severer forms with visceral complications there may be oligocythæmia of extreme grade. v. Jaksch cites a case in which the red count fell from 1,600,000 to 750,000 within three months, and Luzet noted a drop to 500,000 within three weeks (Ewing).

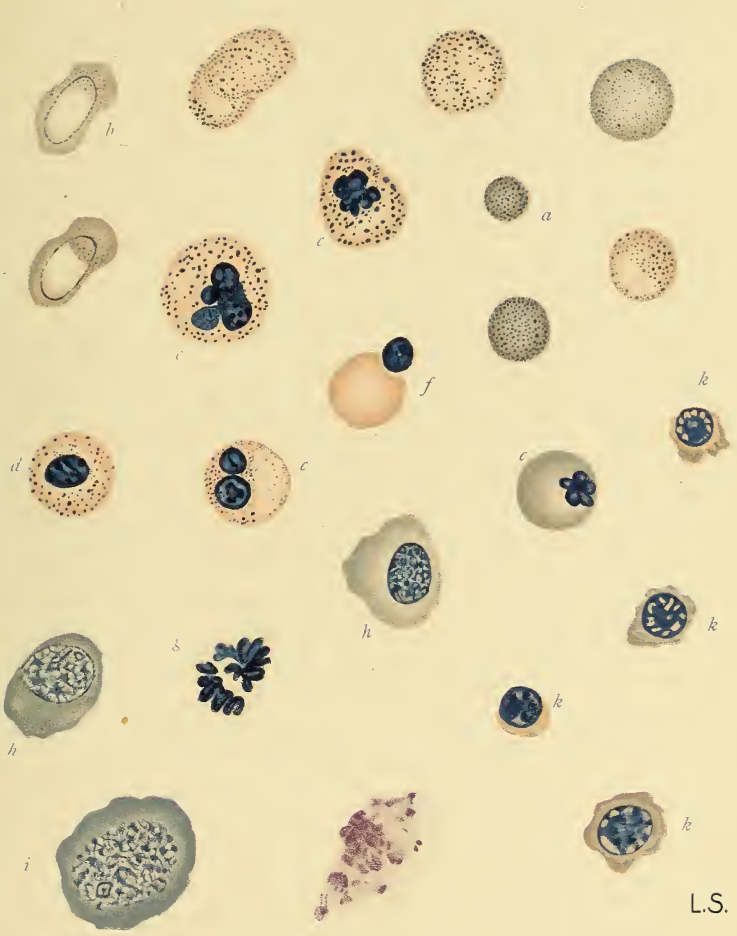
In congenital syphilis the oligocythæmia is usually marked, excepting in very mild cases, and in the severer infections the blood picture may simulate that of pernicious anæmia.

Behavior toward Anilin Dyes.—Polychromatophilia (Polychromasia).—The normal *living* red cell possesses no affinity for dyes; it is achromatophilic. The normal *fixed* cell of the circulating blood, on the other hand, has a marked affinity for acid dyes, such as eosin, orange-G, acid fuchsin, etc.; it is accordingly said to be oxyphilic, and as it takes up only one color from a mixture of different dyes it is termed monochromatophilic. Under various pathological conditions which are associated with a marked grade of anæmia cells are met with which are polychromatophilic. Such cells manifest an affinity not only for acid dyes, but simultaneously also for basic dyes, so that with a mixture of hæmatoxylin and eosin, or eosin and methylene-blue, for example, the red cells are not stained in the usual tint of the hæmoglobin, but present a mixed color in which the tint of the basic dye is more or less apparent. With hæmatoxylin and eosin, for example, some cells will be colored a bluish red, others a reddish blue, still others violet, and again others a pure blue (Plate X.).

As regards the significance of the polychromasia, Ehrlich maintained that the condition was evidence of a degenerative process—of a coagulation-necrosis of the discoplasm—during which this takes

[1] Hühnerfauth, Virchow's Archiv, vol. lxxvi.
[2] Siegel-Maydl, Wien. med. Jahrbuch., 1884.
[3] Rieder, Beit. z. Kenntniss d. Leucocytose, Leipzig, 1892.
[4] Strauss u. Rohnstein, loc. cit.

PLATE III



a, a group of red cells undergoing granular degeneration; *b*, red cells showing Cabot's ring bodies; *c*, normoblasts with nuclei undergoing karyolysis, the bodies of the cells show granular degeneration; *d*, normoblast with pyknotic nucleus; *f*, red cell, suggesting loss of nucleus by extrusion; *g*, red cell undergoing mitosis; *h*, megaloblasts with polychromasia of protoplasm; *i*, gigantoblast; *k*, nucleated red cells with wheel-shaped nuclei undergoing cytolysis; *l*, a group of plaques.

up albumins from the blood-plasma, while at the same time it loses the power of holding its hæmoglobin. As a consequence the oxyphilia diminishes, while owing to the absorption of albumins a more or less well-marked basophilia develops. As a matter of fact polychromatophilia is often seen in cells which are manifestly degenerating, and in myelogenous leukæmia especially one frequently meets with nucleated red corpuscles which are markedly polychromatic and in which the protoplasm is evidently undergoing destruction, often appearing merely as a little hood attached to one side of the nucleus (see Plate III.). Ehrlich accordingly speaks of an *anæmic or polychromatophilic degeneration* of the blood. But, on the other hand, there is evidence to show that polychromasia may be the expression of a regenerative process, and we find as a matter of fact that the erythroblasts of the normal bone-marrow are for the most part polychromatophilic, and the more markedly so the younger they are. Megaloblasts are probably always polychromatophilic (Plate III.). Welker has shown that basophilic red cells are normally found in pigeons, mice, guinea-pigs, cats, and dogs, while they are absent in the horse and the ox. I have also found them in the squirrel. In those animals, moreover, in which the red cells of the circulating blood are normally nucleated a certain grade of polychromasia, according to my experience, appears to be the rule in all the younger cells; the pure hæmoglobin tint is only found in the mature cell and in the oldest forms which are undergoing cytolysis.

Of late, Ehrlich has admitted the existence of a physiological polychromasia, but he still maintains that it may also occur as the expression of a degenerative process.

LITERATURE.—Ehrlich, Charité Annalen, vol. x. p. 136. Engel, Deutsch. med. Woch., 1899, p. 209. Gabritschewsky, Arch. f. exp. Path., vol. xxviii. p. 83; Zeit. f. klin. Med., vol. xxvii. p. 492. Askanazy, Ibid., vol. xxi. p. 415. Maragliano and Castellino, Ibid., vol. xxi. p. 415.

Diabetic Chromatophilia.—Bremer has pointed out that a distinct difference exists in the affinity of diabetic blood for certain anilin dyes, as compared with non-diabetic blood. For, whereas non-diabetic blood is readily stained with Congo-red, methyl-blue, eosin, etc., diabetic blood is distinctly refractory, while such dyes as Biebrich-scarlet, which readily stain the diabetic blood, do not color non-diabetic blood. Upon this peculiarity in the behavior of the red corpuscles *Bremer's diabetic blood test* is based.

METHOD.—A drop of blood of moderate size is mounted on a slide and spread out in a wave-like manner, using the edge of a second slide for this purpose. A number of such preparations are made, as also an equal number with normal blood for control. These are then placed on the tray of a drying-oven at a distance of 12 cm. from the bottom. The bulb of the thermometer is fixed

at the same level. The temperature is then rapidly raised to about 130° C., when the flame is removed. Care should be taken that the temperature thereafter does not exceed 140° C.; the optimum lies at about 135° C. The apparatus is then allowed to cool until the preparations can be conveniently handled, when a specimen of the diabetic blood is placed back to back with a control-specimen, and both are immersed in the staining fluid. A 1 per cent. aqueous solution of Congo-red, which should always be made up freshly when required, is advantageously employed. After exposure for from one and a half to two minutes the specimens are rinsed in water and dried with filter-paper. It will then be seen that the non-diabetic blood is stained the color of Congo-red, while the diabetic blood is either not stained at all or presents merely an orange color.

Other stains may also be employed, such as a 1 per cent. aqueous solution of methyl-blue or Biebrich-scarlet, or Ehrlich's tri-acid stain, the eosinate of methylene-blue, and others. When using methyl-blue analogous results are obtained as with Congo-red. With Biebrich-scarlet, on the other hand, the diabetic blood takes up the color, while the non-diabetic specimen proves refractory. If Ehrlich's stain is employed, an exposure to the stain for from two to five minutes is necessary; the diabetic specimen is stained orange, the non-diabetic blood violet.

Very satisfactory results are obtained also with the following method: the preparations are first stained for from one and a half to two minutes in a 1 per cent. aqueous solution of methyl-green. Upon washing, it will be seen that both specimens are colored green, but the diabetic blood more markedly so than the other. Both are then immersed for from eight to ten seconds in a 0.12 per cent. aqueous solution of eosin, when the diabetic blood remains green, while the non-diabetic specimen is colored eosin. Analogous results are obtained with methylene-blue and eosin.

Success in these examinations depends essentially upon the proper degree of temperature during the process of fixation. But care should also be had not to leave the specimens in the staining solution longer than indicated, and to rinse quickly in water and dry.

Regarding the nature of the substance in diabetic blood which is responsible for this peculiar behavior, little is known, but it appears certain that the reaction is not dependent upon the presence of glucose nor upon the degree of alkalinity of the blood, as suggested by Lépine and Lyonnet. Bremer's claim that the reaction is pathognomonic of diabetes and glucosuria, and may even yield positive results in the pre-diabetic stage of the disease, and when the sugar has temporarily disappeared from the urine, has been confirmed in all essential points, both in this country and abroad. A few interesting exceptions, however, have been noted. In animals, for

example, in which glucosuria has been artificially produced by means of phlorhizin, the reaction does not occur, whereas in phloroglucin-diabetes positive results are obtained. In Bremer's entire series of diabetic cases a negative result was obtained but once, and in this instance he believes that the diabetes was of the renal type, and analogous to the phlorhizin-diabetes of animals. He suggests that it may thus be possible to differentiate this form from the hæmatogenic variety, using the latter term in its widest sense. Lépine and Lyonnet report a positive result in one case of leukæmia, but Bremer believes this to have been due to faulty technique. Hartwig finds that Bremer's reaction is constant in diabetes, but that it may also occur at times in other conditions.

LITERATURE.—L. Bremer, "An Improved Method of Diagnosticating Diabetes from a Drop of Blood," N. Y. Med. Jour., 1896; Centralbl. f. inn. Med., 1897, p. 521. Le Goff, React. chrom. du sang diabet., Paris, 1897. Lépine and Lyonnet, Lyon méd., vol. lxxxii. p. 187. Hartwig, Deutsch. Arch. f. klin. Med., vol. lxii. p. 287.

Granular Degeneration of the Red Cells.—Under pathological conditions red cells may be met with which contain basophilic granules. These are readily stained with methylene-blue, methylene-azure, thionin, etc. Methyl-green, however, which is a specific nuclear dye, does not stain the granules. Their size, form, and number are variable. While the majority are round, others are rod- or biscuit-shaped. The largest granules are found in pernicious anæmia and in cases of lead-poisoning with intestinal manifestations. They are then quite readily seen and attract attention at once (Plate III.). In most other diseases in which they occur they are much smaller, and on superficial examination they may indeed be overlooked; some cells at first sight merely look a little off-color, and it is seen only on very careful examination that the apparent polychromasia is in reality due to the presence of large numbers of minute dots. This finest variety is in my experience most commonly seen in obscure cases of lead-poisoning, or in such cases which do not present intestinal symptoms. In other conditions the size is about that of a neutrophilic granule. Very often, in specially anæmic cells, the granules are arranged in the peripheral portion of the cell. Their number is exceedingly variable; generally speaking, it depends upon their size; when they are especially large they are relatively little numerous.

The granules may occur in cells of normal size or color, in poikilocytes, and in nucleated red cells, both of the normoblastic and the megaloblastic type, especially the former. Not infrequently they are seen in cells which are markedly polychromatic, but, like Grawitz, I do not believe that granular degeneration represents a later phase of polychromasia.

In disease they are most constant and numerous in pernicious anæmia, in lead-poisoning, and in malaria; they are less constant

and less numerous in the leukæmias, in pseudoleukæmia, in the cachexias referable to septic infection, syphilis, carcinomatosis, and in the final stages of tuberculosis. In chlorosis and in the anæmia of chronic nephritis they are absent; in a case of v. Jaksch's anæmia, in which nucleated red cells were quite numerous, I obtained negative results.

In pernicious anæmia granule cells are frequently found in the interval and at a time when the blood picture is otherwise practically normal. I have seen them most numerous in a case in which blood crises occurred from time to time (see page); almost every normoblast contained granules; non-nucleated granule cells were, however, at the same time present in large numbers.

In lead-poisoning granule cells are practically found without exception, and may be encountered at a time when no clinical symptoms are manifest. The amount of lead which is necessary to call forth their appearance is quite small, and it is a common experience to meet with a small number after the administration of lead in medicinal doses. I have found them after the ingestion of only 0.5 gramme given in divided doses in the course of forty-eight hours. In cases of lead-poisoning they persist for a long time after exposure has ceased. In one case of double wrist- and ankle-drop I could still demonstrate granule cells after five months.

In malaria granule cells are very common. Plehn found them in Europeans after a short sojourn in the tropics, and looked upon the granules as spores of the malarial parasite.

In septic cases and in the cachexia of carcinomatosis they are not numerous; in a case of cancer of the stomach with only 27 per cent. of hæmoglobin, which I recently observed, I found no granule cells.

In the early stages of phthisis granular degeneration is not seen, but it may occur later, when a general septicæmia has supervened.

As regards the significance of the granules, Engel, Ehrlich, and others have suggested that they are most likely products of karyolysis. But Grawitz, Stengel, Pepper, and White maintain, and I think rightly so, that they are not of nuclear origin. They may be found at a time when not a single nucleated red cell is demonstrable in the blood; nucleated red cells may be seen in which no sign of karyolysis is manifest, while the body of the cells is studded with granules; unlike the nuclei of the erythroblasts, the granules have no affinity for methyl-green, which is a specific nuclear dye; they may be found in nucleated cells which are undergoing karyokinetic division.

It is interesting to note that granule cells may not be found in the bone-marrow even when they are numerous in the circulating blood; when they do occur, they are not more numerous than in the peripheral vessels. Grawitz hence regards their presence as an indication of a degenerative change in the hæmoglobin, and speaks of the phenomenon as "granular degeneration." Schmauch, on the

other hand, has observed similar appearances in the blood of healthy cats, and Engel has described the occurrence of granule cells in the blood of early cat embryos. I have found granule cells in the squirrel and in a porcupine suffering from filariasis. It is stated by all observers that they do not occur in the blood of man when in perfect health. My own experience has practically been the same, but I have found small numbers in two individuals who at the time considered themselves perfectly well. One of these at the same time had 6.5 per cent. of eosinophiles and had recently passed a round worm.

In white mice Grawitz was able to produce a granular degeneration by prolonged exposure of the animal to a temperature of from 37° to 40° C. He suggests that the analogous results which were obtained by Plehn in the case of Europeans after a brief sojourn in the tropics may possibly be referable to the high temperature.

LITERATURE.—E. Grawitz, "Ueber Körnige Degeneration d. rothen Blutzellen," Deutsch. med. Woch., 1899, No. 36, p. 585; "Klinische Bedeutung u. experiment. Erzeugung körniger Degenerationen," etc., Berlin. klin. Woch., 1900, p. 181; "Granular Degeneration of the Erythrocytes," etc., Am. Jour. Med. Sci., 1900, vol. cxx. p. 277. Bloch, Deutsch. med. Woch., 1899, V. B. p. 279. Litten, Ibid., No. 44. Behrendt, Ibid., No. 44. White and Pepper, "Granular Degeneration of the Erythrocyte," Am. Jour. Med. Sci., 1901, vol. cxxii. p. 266. C. E. Simon, International Clinics, 1902, vol. i. p. 69. Stengel, White, and Pepper, Am. Jour. Med. Sci., 1902, vol. cxxiii. p. 873.

Cabot's Ring Bodies.—Cabot has recently drawn attention to the occasional occurrence in red cells of curious ring bodies which are usually stained red with Wright's modification of Leishman's stain, but which may also take on a blue color. He found such rings in three cases of pernicious anæmia, in three of lead-poisoning, and in one case of lymphatic leukæmia. I have been able to demonstrate the same structures with the eosinate of methylene-blue, and could verify Cabot's observation that they occur in granule cells, but may also be found in apparently normal red corpuscles (Plate III.). No doubt they bear some relation to the nucleoids.

LITERATURE.—Cabot, Jour. Med. Research, 1903, vol. ix.

Ehrlich's Hæmoglobinæmic Innenkörper.—These structures may be encountered in red cells in conditions associated with extensive hæmocytolysis the result of specific blood poisons. The individual body is round and characterized by its affinity for acid dyes.

Nucleated Red Corpuscles.—Nucleated red corpuscles are not found in the circulating blood of healthy individuals, excepting at birth and during the first days of life, when it is not unusual to meet with an occasional cell of this type. In the bone-marrow, however, they are always found. It is here possible to distinguish two types, viz., the normoblast and the megaloblast. The latter is ontogenetically the older and gives rise to the normoblast through a process of homoplastic differentiation by cell division; it thus bears the same relation to the normoblast which exists between the large

lymphocyte and the small lymphocyte, and the amblychromatic myelocyte and the trachychromatic myelocyte (see page). The megaloblast itself results from the large lymphocyte through direct heteroblastic transformation and ages into the macrocyte, while the normoblast similarly develops into the normocyte (Pappenheim). In the bone-marrow of adults megaloblasts are only found in small numbers; the great majority of nucleated red cells are of the normoblastic type.

The **normoblasts** (Plate III.), like the normal red cells of the circulating blood, have a diameter which varies from 6 to 9 μ. The nucleus in the youngest cells occupies a central position, and is larger and relatively poorer in chromatin than in the older cells, where it is frequently located excentrically. The size varies between 2 and 4 μ. In the younger cells the chromatin is quite commonly arranged in a stellate manner, while later the nuclear juice manifestly diminishes in amount, and in the oldest cells the nucleus is accordingly much smaller and markedly pyknotic; but in all cells it stains quite deeply.

The protoplasm in the majority of the normoblasts of the bone-marrow is polychromatophilic, while in most of the cells which are found in the peripheral blood in disease the oxyphilic tendency prevails. But at times here also the normoblasts are polychromatophilic. With the triacid stain a few cells of this type, in the bone-marrow, take on the fuchsin stain (Engel's fuchsinophilic cells).

In cases of pernicious anæmia, in myelogenous leukæmia, and in lymphatic leukæmia (notably the acute form) normoblasts are at times seen which are undergoing mitosis (Plate III.).[1] Much more common, however, are cells in which the nucleus is more or less lobed, and presents appearances which are commonly interpreted as evidence of beginning karyolysis (Plate III.). Free nuclei may likewise be seen in the blood.

In the majority of cases in which normoblasts are found in the blood these are well preserved, but in myelogenous leukæmia more especially it is very common to meet with cells in which the protoplasm surrounding the nucleus is much diminished in amount and presents a ragged outline. These cells are manifestly degenerating, and in many specimens the protoplasm will be seen reduced to a little hood which is attached to one side of the nucleus (Plate III.). Such cells in my experience are always polychromatophilic and are apt to be mistaken by the beginner for poorly stained lymphocytes.

The occurrence of normoblasts in the circulating blood is always evidence of stimulation of the bone-marrow, which may occur either indirectly, as the result of an "anæmic" condition of the blood, or directly, as in disease of the bone-marrow *per se* (Grawitz). We may accordingly meet with normoblasts in almost any form of

[1] G. Dock, "Mitosis in Circulating Blood," Trans. Assoc. Am. Phys., 1902, p. 484.

anæmia, be this the result of traumatism (post-hemorrhagic), of inanition, or of organic disease. In the acute forms of anæmia they are apt to be most numerous, but even in the more chronic cases and in cachectic conditions specimens of blood may be obtained in which one or more normoblasts are seen in every field. In the secondary anæmias, however, it is usual to meet with only a few nucleated cells.

At times there appears to occur a sudden invasion of the circulating blood by red cells, many of which are nucleated; this phenomenon v. Noorden terms a *blood crisis*, and it is noteworthy that the invasion of the red cells may be preceded and accompanied by a very extensive increase of the leucocytes. Ehrlich cites a case of hemorrhagic anæmia, reported by v. Noorden, in which at the time of such a blood crisis the normoblasts were so numerous, while hyperleucocytosis of high grade existed at the same time, that the blood condition strongly suggested the existence of a myelogenous leukæmia. The increase of the red cells in this case amounted to almost double their original number.

To estimate the extent of a blood crisis, the following examinations are necessary:

a. A determination of the absolute number of red corpuscles.

b. A determination of the ratio between the white and red cells.

c. A determination of the ratio between the nucleated red and white cells, in dried specimens, by the aid of a quadratic ocular diaphragm.

EXAMPLE.—Supposing that in a given case 3,500,000 red corpuscles are found in the cbmm., while the ratio of the white to the red corpuscles is 1 : 100, and that of the nucleated red to the white 1 : 10; 3500 nucleated red corpuscles must hence be present in each cbmm. of blood—*i. e.*, 1 for each 1000 of normal red corpuscles.

From the standpoint of prognosis it is noteworthy that a fatal issue may be expected whenever the number of red cells falls below 1,500,000 and normoblasts are absent.

The Megaloblasts.—These are usually from two to three times as large as the normoblasts, but may attain even more extensive proportions (Ehrlich's gigantoblasts). They are provided with a relatively large centrally located nucleus, which is wide-meshed and which with the triacid stain is not colored nearly so deeply as the normoblastic nucleus. In some specimens, indeed, the affinity for methyl-green is so little marked that at first sight a nucleus can hardly be distinguished. With those staining mixtures, on the other hand, which contain methylene-blue as base, it can always be fairly well made out. But owing to the fact that these cells are almost invariably polychromatophilic, the nucleus may at first be overlooked, as the polychromatic protoplasm appears in the meshes of the nucleus and

sometimes differs but little in color from the chromatin. The inexperienced not infrequently mistake such cells for large mononuclear leucocytes that are somewhat off-color; the character of the nucleus, however, viz., its wide meshwork, should prevent this mistake.

Mitoses in megaloblasts are also at times seen.

Under normal conditions a few megaloblasts may be found in the blood of very young infants, and it is noteworthy that in the severe types of secondary anæmia megaloblasts are far more apt to occur in children than in adults. But even then they are rare. According to Ehrlich, the presence of megaloblasts is evidence of a reversion of blood formation to the embryonic type and of grave prognostic import. He regarded their presence as indicative of essential pernicious anæmia; and, as a matter of fact, they are here quite constantly met with and represent one of the most important features of the disease. They are rarely numerous, however, and there are cases in which they do not occur in the circulating blood. A few cases of pernicious anæmia have indeed been reported in which neither megaloblasts nor normoblasts could be demonstrated at any time (Ehrlich, Lazarus, Engel, Pane[1]).

The modern tendency is to regard the appearance of megaloblasts in the blood as evidence of an anæmia of unusual severity, and not as an indication of any one disease or of malignant degeneration. While they are undoubtedly most constant in pernicious anæmia, they may also be met with in other forms. They have been found in leukæmia, in lead-poisoning, and even in chlorosis. I have seen a few megaloblasts in v. Jaksch's pseudoleukæmia infantum, and in a case of severe infantile anæmia referable to amœbic colitis. In cancer of the stomach, according to Osler and McCrae, they are rarely if ever found. Askanazy[2] has reported an interesting case of bothriocephalus infection in which the megaloblastic type of blood regeneration disappeared after expulsion of the parasites—sixty-seven in number—and was replaced by the normoblastic type, the case ending in recovery.

In cases of traumatic anæmia unusually small nucleated red cells have at times been observed. These are termed *microblasts*. They have attracted but little attention and are quite rare. I have seen such cells, measuring not more that 3–3.5 μ, in a case of pernicious anæmia at the time of the blood crisis, in which large numbers of normoblasts were also present.

[1] Pane, "Sull' anemia progressiva mortale senza corpuscoli rossi, nucleati nel sangue," Riform. med., 1900, No. 263.

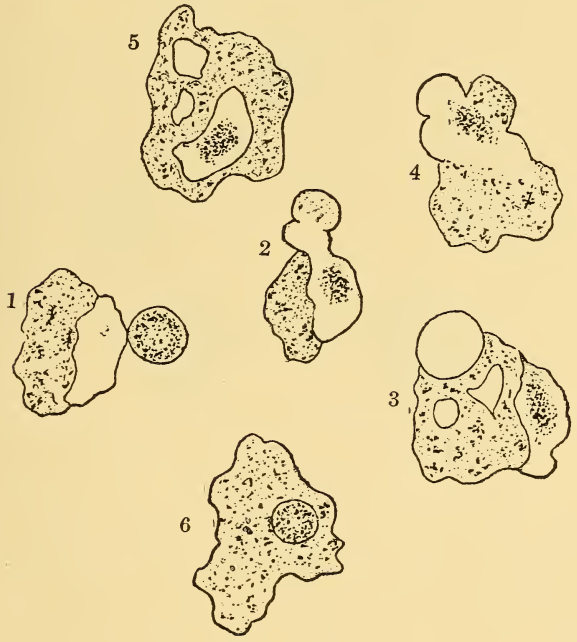
[2] Askanazy, Zeit. f. klin. Med., 1895, vol. xxvii.

The Leucocytes.

General Characteristics.—The leucocytes, or white corpuscles of the blood, as seen in the wet preparation (Plate II., Fig. 1), are roundish or irregularly shaped cells, which vary in size, but for the most part are larger than the red corpuscles. They are all nucleated, and, as the term indicates, devoid of coloring-matter. In a general way they may be divided into two distinct classes, viz., those which are granular and those which are not granular.

The *granular cells* are by far the most numerous, and are characterized by the fact that they are capable of active locomotion. Even without a warm stage it is almost always possible to observe this in the ordinary wet preparation. The moving cells at once attract

FIG. 14.



Phagocytosis.

attention by their irregular outline. On careful examination with a high power it will then be noted that the cell advances in a definite manner, which is quite analogous to what is seen in the amœba. The protoplasmic portion of the cell manifestly consists of two parts, viz., a non-granular hyaline ectosarc and a granular endosarc. As the leucocyte progresses the hyaline ectosarc advances with a flowing motion, forming a distinct layer in front of the granular endo-

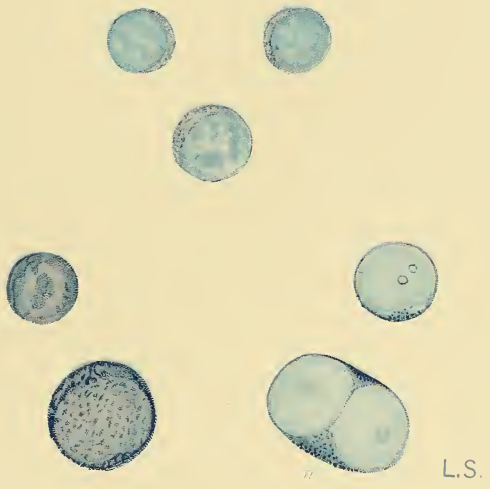
sarc, which itself then merges into the non-granular portion. The moving leucocyte is roughly pear-shaped, with the base in advance, while the rear end tapers markedly and frequently seems to drag behind it a small roundish mass which, like the main body of the cell, is also granular. These granular leucocytes are the so-called *phagocytes* of Metchnikoff, so termed from the fact that, like amœbæ, they take up foreign matter into their interior. This can be demonstrated especially well in malarial blood taken at the time of a paroxysm, when extracellular organisms (page 188) are present. Placing one of these in the centre of the field, it may be observed how one or more leucocytes will gradually approach the organism and finally engulf it, as shown in the accompanying illustration (Fig. 14). According to Metchnikoff, the phagocytic function is the most important function of the leucocytes, and the outcome of a bacterial invasion, figuratively speaking, will depend upon the superiority of the organisms engaged in warfare.

The nucleus of the granular leucocytes is polymorphous—*i. e.*, it is composed of different lobes which in life are joined together, while in dead cells the impression is gained as though a number of nuclei existed; this fact accounts for the earlier but more common term *polynuclear*, instead of *polymorphonuclear leucocytes*.

While the granules in the majority of the leucocytes are very fine (Plate II., Fig. 1), on careful search some cells will be found in which they are very coarse and highly refractive. This coarsely granular variety is very characteristic in appearance and at once attracts attention. The cells are far less numerous, however, and as a matter of fact represent only from 1 to 4 per cent. of the total number of the leucocytes, while the finely granular variety represents from 60 to 70 per cent.

The *non-granular leucocytes*, in contradistinction to the granular variety, are all mononuclear. They are quite hyaline in appearance, and are readily overlooked by the beginner unless a somewhat subdued light is used in the examination. Two varieties may be recognized: one about the size of a red corpuscle, the other somewhat larger. The nucleus in both varieties occupies a considerable portion of the cell and is surrounded by a layer of protoplasm which is practically hyaline. Every cell, it is true, contains a few granules collected at a certain point along the periphery, where the protoplasm is more extensively developed than elsewhere; but these granules, in contradistinction to those which we see in the polynuclear varieties, probably represent nodal points in the cytoreticulum, and not a specific secretory product, as which Ehrlich and his school view the granules of the polynuclear variety. In the small mononuclear form one or sometimes two small brownish granules can also usually be discerned somewhere in the peripheral layer of the protoplasm. Of the significance of this granule, so far as I am aware,

PLATE IV.



Lymphocytes.

The cell *a* shows nucleus after division, each with a nucleolus.

nothing is known, nor has its presence been previously described (see Plate II., Fig. 1).

The non-granular mononuclear leucocytes, in contradistinction to the polynuclear granular variety, were formerly regarded as non-motile. Jolly, Wolff, and others have recently shown, however, that they also are capable of changing their form even though progressive locomotion may not occur. The change in form can readily be demonstrated even without a warm stage, and it will be observed that the nucleus takes an active part in these changes. Heinen demonstrated this in my laboratory without having knowledge of Wolff's paper, which then had just appeared.

Classification.—While it is possible to distinguish several varieties of leucocytes in the wet and unstained preparation, a more complete picture of the structure of the individual forms may be obtained from a study of specimens that have been variously stained with acid, basic, and neutral anilin dyes (see page 119). The study of such preparations, moreover, forms the most satisfactory basis of classification of the different forms of leucocytes which we possess at the present time. We distinguish the following varieties:

1. **The Lymphocytes (Small Mononuclear Leucocytes)** (Plate IV.).—The lymphocytes which occur normally in the blood are for the most part a little smaller than the red corpuscles or of equal size. The nucleus is single and surrounded by a narrow rim of protoplasm which is generally described as non-granular. But, as I have pointed out, a few granules can almost always be made out in the wet preparation at a certain point along the periphery where the protoplasm is a little more extensively developed. These granules, however, probably represent nodal points of the cytoreticulum, and are not to be regarded as in any way analogous to the granules which are met with in the polynuclear leucocytes. Nucleus and protoplasm are both basophilic, and, generally speaking, the protoplasm is so more markedly than the nucleus. This is best seen in specimens that have been stained with methylene-blue, where the lymphocytes for the most part present a comparatively feebly staining nucleus which is surrounded by a rim of dark blue. Other cells belonging to the same group, however, will also be seen in which this is not marked, but in which the staining affinities of both nucleus and protoplasm appear about the same. These cells are generally a little larger than the first variety, with a somewhat broader zone of protoplasm and an excentric position of the nucleus. We may speak of them as *medium-sized lymphocytes* in contradistinction to the small-sized variety. A still larger form may also be met with, but it is not commonly seen under normal conditions except in children. The staining properties of these *large lymphocytes* are essentially the same as those of the smaller varieties; in some the protoplasm is more intensely basophilic than the nucleus, while in others the

basophilia of both is about the same. The position of the nucleus may be either concentric or excentric, as in the smaller forms. This large type is notably seen in acute lymphatic leukæmia, but it also occurs in the chronic form.

With certain dyes, like methylene-blue, the protoplasm of the lymphocytes does not appear perfectly homogeneous, but presents a peculiar granular appearance. This is referable to nodal points of the cytoreticulum and does not represent a true granulation. With methyl-green, and hence with Ehrlich's triacid stain, the protoplasm is perfectly homogeneous and appears as a pale rim about the somewhat more deeply staining nucleus. While it is thus impossible with the usual dyes to demonstrate the existence of a true granulation in the lymphocytes, Michaelis[1] has called attention to the fact that with eosin-methylene-azure solutions (page 123) distinct granules can be seen. Their significance, however, has not as yet been established. Very curiously these granules could not be demonstrated in the lymphocytes obtained from the lymph-glands directly, and it appears that they are present in only a certain percentage of those occurring in the blood. The number of granules in a cell is variable; in some only two or three are seen, while in others the protoplasm is literally studded with them. Their size varies between that of the common neutrophilic and that of the eosinophilic varieties.

In wet specimens, as I have pointed out, one or two reddish-brown granules are quite commonly seen in most of the lymphocytes. In stained preparations these cannot be demonstrated.

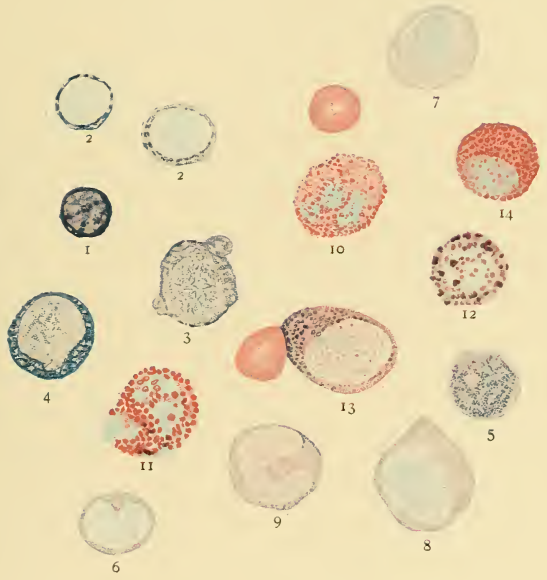
The outline of the cell in the smaller forms is usually fairly smooth, but in the larger varieties it is often shaggy, and at times specimens are seen with a number of distinct knobs. These manifestly represent bits of protoplasm which are being constricted off, a process which can be directly observed in the wet preparation. In the dried and stained specimen also it is not uncommon to meet with such particles occurring free in the blood, the origin of which is quite manifest from their staining properties, which are the same as those of the parent cell.

The nucleus, in the smaller forms especially, is concentrically located, while in the larger varieties, in which the protoplasm is more extensively developed, it commonly occupies an excentric position. In the stained specimens, especially in the larger cells, it is sometimes surrounded by a faint areola, which is probably owing to artificial retraction. The nucleus is more commonly oval or bean-shaped than round; deep invaginations are not often seen and fragmentation of the nucleus is rare. Such cells present an appearance which is altogether different from that of the true polynuclear elements.

Lymphocytes undergoing mitosis are sometimes seen in the blood

[1] Michaelis and Wolff, Virchow's Archiv, 1902, vol. clxvii. p. 151.

PLATE V.



Note the size of the various leucocytes, as compared with the red corpuscles at 15. Figs. 1, 2 and 6 represent the most common forms of the small type of lymphocytes; 3 and 5 belong to the same group, but are manifestly atypical; 3 shows the knob-like projections, described in the text; 4 represents the large type of the lymphocyte, and shows the vacuolated appearance of the protoplasm, which is so commonly seen. The metachromatism of the protoplasm, however, does not appear here as in nature. 7 and 8 are representatives of the large variety of mononuclear leucocytes; 9 may be classed as a transition form, which is as yet devoid of granules; 13 represents a neutrophilic myelocyte, 14 an eosinophilic myelocyte, 10 a neutrophilic polynuclear leucocyte, 11 an eosinophile of the same type, and 12 a typical basophilic leucocyte.

The preparations were stained with the eosinate of methylene-blue and drawn to scale. (Bausch and Lomb, Eye-piece 1 inch, objective 1-12th.)

of lymphatic leukæmia. Characteristic figures, however, are comparatively rare, and it is more common to meet with cells in which division of the nucleus has already occurred (Plate IV.). Nucleoli are not usually seen in the lymphocytes of the normal blood, and seem to be comparatively infrequent also in the blood of lymphatic leukæmia. Occasionally, however, specimens are met with in which they are quite distinct, and at the same time multiple ; in such cases active cell-division seems to take place in the circulating blood.

The reaction of the protoplasm of the lymphocytes, as tested with the erythrosin method (page 139), is strongly alkaline. The cells contain no glycogen.

In adults the number of the lymphocytes normally varies between 20 and 30 per cent. Higher values are found in young children, especially during the first year of life, when the lymphocytes constitute from 50 to 60 per cent. of the total number. At birth, however, they are curiously less numerous than in adult life, viz., only about 15 to 16 per cent. They very soon increase, however, and by the twelfth day it is usual to have from 40 to 50 per cent. After the fifth year adult values are normally the rule.

In disease the number of the lymphocytes may be increased or diminished, conditions which are spoken of respectively as *lymphocytosis* and *lymphopenia* (see pages 105 and 107).

While it was formerly supposed that the lymphocytes originate only in the lymph-glands proper, there is evidence that they may be formed wherever there is lymphoid tissue, and hence also in the spleen and in the bone-marrow. They are probably derived from the so-called large lymphocytes of the germinal centres indirectly through a process of differentiating karyokinesis, and represent fully differentiated cells which are incapable of further development.

The large lymphocyte itself, as I have pointed out, practically occurs in the blood only under pathological conditions. According to Pappenheim's views, it represents the *Ur* or *Stammzelle*, from which all other forms of leucocytes are directly or indirectly derived. The large lymphocytes are identical with Benda's lymphogonia, Troje's lymphoid marrow-cells, Nägeli's myeloblasts, and the undifferentiated lymphoid cell of Michaelis and Wolff.

2. **The Large Mononuclear Leucocytes** (Plate V.).—These are mostly two or three times as large as the red corpuscles and provided with a large single nucleus, which is surrounded by a relatively wide zone of non-granular protoplasm. The nucleus in some cells is oval or elliptical, while in others it is more or less invaginated. In the past it has been customary to classify large mononuclear leucocytes with karyolobic nuclei under a special heading—the *transition-forms* of Ehrlich—and it was supposed that a certain number of large mononuclear leucocytes aged directly in the circulating blood into the polynuclear neutrophilic leucocytes with the transition-form as

an intermediary stage. This view, however, is open to many objections and there are good grounds for the present tendency to regard the transition-form merely as the mature form of the large mononuclear leucocyte and as incapable of further differentiation (Pappenheim).

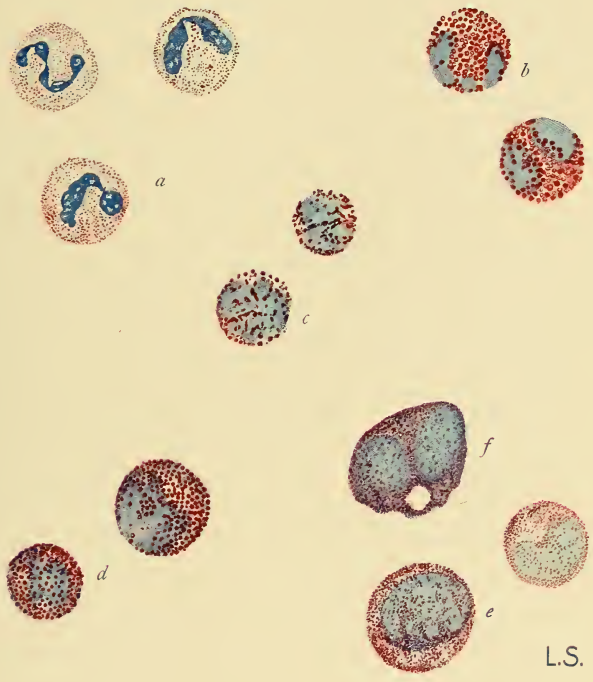
In the wet preparation the large mononuclear leucocytes are exceedingly hyaline, so that they are readily overlooked by the beginner. Both nucleus and protoplasm are basophilic, but much less markedly so than the lymphocytes, and it is noteworthy that the protoplasm usually possesses a less marked affinity for the basic dye than the nucleus. Cells, however, are also met with in which the affinity for the dye is about the same in both structures. If by chance this occurs in specimens which are somewhat smaller than usual, a certain amount of difficulty arises in differentiating such small "large" mononuclear leucocytes from certain lymphocytes, in which the staining reaction is also not typical. A hard-and-fast line of distinction cannot here be drawn, and in every differential leucocyte count the personal equation will of necessity enter into consideration. The salient characteristics of the two types should, however, be borne in mind; in the lymphocytes the protoplasm is but feebly developed in relation to the size of the nucleus, while in the large mononuclear leucocyte the reverse holds good. The protoplasm in the latter, moreover, is apparently much more delicate in structure, and is readily wrinkled by contact with adjacent cells; not infrequently cells of this type are found which have manifestly been torn or otherwise injured during the preparation of the specimen, while the lymphocytes are usually well preserved.

In preparations that have been stained with Ehrlich's triacid both nucleus and protoplasm are very faintly colored and the latter appears perfectly homogeneous; but in specimens which have been stained with mixtures containing methylene-blue as the basic component, the protoplasm presents a somewhat granular appearance, which, as in the lymphocytes, is referable to the existence of a cytoreticulum. A certain proportion of the large mononuclear leucocytes (including the transition-forms), however, also contain granules which, as in the case of the lymphocyte, can be stained with eosin-methylene-azure solutions.

Inclusive of the transition-forms the large mononuclear leucocytes normally represent from 1 to 6 per cent. of the total number. They are relatively more numerous in young children, in whom the highest values are found between the sixth and ninth day after birth. Many of the cells at this time are of the type of the transition-form; they may number 18 per cent.

According to Ehrlich, the large mononuclear leucocytes originate in the bone-marrow and possibly also in the spleen. As Pappenheim has suggested, they may develop directly, cytogenetically, from the

PLATE VI.



Granulocytes.

a, polynuclear neutrophilic leucocytes; *b*, polynuclear eosinophilic leucocytes; *c*, mast cells; *d*, young eosinophilic myelocytes; *e*, neutrophilic myelocytes; *f*, the nucleus here has just undergone division: the clear space is a vacuole.

"large" lymphocytes, and then age into the transition-form, which represents the final stage in the development of this type. The former view, according to which the large mononuclear leucocyte develops directly cytogenetically from the small lymphocyte, has practically been abandoned.

3. **The Neutrophilic Polynuclear Leucocytes** (Plate VI.).—These cells are a little smaller than the large mononuclear leucocytes and the transition-forms and represent the finely granular variety, already referred to. They are the phagocytes, *κατ' ἐξοχήν*, and as such capable of most active progressive locomotion. The nucleus is a long body, which is commonly twisted upon itself in various ways, so as to resemble the letters S, Y, E, and Z. Often also it is broken up into fragments, suggesting the presence of several nuclei, which accounts for the original designation of these forms as *polynuclear leucocytes.* As Ehrlich has suggested, however, the polynuclear appearance is probably referable to post-mortem changes, the condition of the nucleus being in reality polymorphous. They are accordingly also spoken of as *polymorphonuclear neutrophilic leucocytes;* but the older term, though not so correct, is the more common. The nucleus stains very readily with all nuclear dyes; it is coarsely reticulated and generally shows evidence of a central nodal thickening in each one of its lobes.

The protoplasm proper has a marked affinity for most of the acid dyes; its reaction with the erythrosin method is alkaline, but less so than in the lymphocytes and large mononuclear leucocytes. Under normal conditions a glycogen reaction is not obtained.

Embedded in the protoplasm are numerous fine granules—the ε-granulation of Ehrlich—which are characterized by their affinity for neutral dyes. Hence the term polynuclear *neutrophilic* leucocytes. These granules are ordinarily very abundant; but in disease they may diminish in number until very few are left, and in certain cases of chronic leukæmia they may indeed be absent altogether. Ewing[1] has called especial attention to the decrease in the number of the granules in the acute leucocytoses; this decrease, in my experience, is even more marked in the chronic cases, and especially so in the septic infections. Associated with the diminution in the number of the granules there are very frequently also certain degenerative changes affecting the nuclei. These may be of the type of karyolysis with swelling and loss of chromatin, or of karyorhexis with hyperchromatosis and fragmentation of the nucleus. The former is the more usual in the acute leucocytoses, while the latter is seen especially in leukæmia. In cases of the myelogenous variety it is here quite common to note complete fragmentation of the nucleus into six to ten segments. This phenomenon was first observed by Ehrlich in a case of hemorrhagic smallpox, and is of

[1] Ewing, Clinical Pathology of the Blood, Lea Bros., 1st ed., p. 113.

common occurrence in fresh exudates. Cell degeneration associated with loss of chromatin and swelling, while it no doubt occurs to a greater degree in disease, may also be observed under normal conditions. In every dried and stained specimen a certain number of such cells will be found in which the nucleus appears as a much swollen and but faintly staining shadow, the *Kernschatten* of the Germans, sometimes surrounded by some of the granules, which appear scattered as though the cell had been burst asunder by force; at other times the Kernschatten alone remains and nothing is seen of the body of the cell.

I have stated that the loss of granules on the part of these cells may go on to a point where they are absent altogether. It may happen, however, that the granules are only apparently absent, and merely do not react as usual with ordinary dyes. A proper explanation of this peculiar behavior cannot be given, but every worker in blood is no doubt familiar with the phenomenon. Sometimes a change in the mode of fixation will cause the granulation to appear; at other times it may be demonstrated by the aid of some other dye.

Vacuolization of the polynuclear leucocytes is very much less common than in the case of the mononuclear elements.

Neusser[1] some years ago called attention to the fact that with a certain modification of Ehrlich's triacid stain it is possible to demonstrate the presence of basophilic granules about the nucleus of some of the polynuclear leucocytes, as well as the mononuclear elements. He, as well as Kolisch,[2] regarded the presence of these *perinuclear* granules as characteristic of the so-called uric acid diathesis. As tubercular disease, moreover, is usually not seen in such cases, Neusser thought the presence of these granules in cases of phthisis to be a favorable symptom. Futcher,[3] on the other hand, was unable to confirm these observations, and my own investigations[4] are likewise opposed to Neusser's conclusions. I was able to demonstrate the granules both in health and disease in almost every case, and was at one time even led to think that their absence was of more significance than their presence. A relation between their presence and the elimination of uric acid or xanthin bases certainly does not exist. Within recent years the subject has received no further attention, especially since Ehrlich expressed the belief that the granules are artefacts. He states that they are only exceptionally seen when solutions of chemically pure crystalline dyes are used, from the Actiengesellschaft für Anilinfarbstoffe in Berlin.

The neutrophilic granulation, in contradistinction to the eosinophilic granulation and the mast-cell granulation, does not occur generally distributed among vertebrate animals, but, according to

[1] Neusser, Wien. klin. Woch., 1894, p. 71.
[2] Kolisch, Ibid., 1895, p. 797.
[3] Futcher, Johns Hopkins Hosp. Bull., May, 1897.
[4] Simon, Am. Jour. Med. Sci., vol. cxvii. p. 139.

Ehrlich, is found only in man and the ape. My own researches in this direction tend to confirm Ehrlich's views, which have been repeatedly assailed from several directions. Granulations of this character which are not common to all animals are termed *special* granulations.

The polynuclear neutrophilic leucocytes are derived from corresponding mononuclear forms—the neutrophilic myelocytes—which are normally found only in the bone-marrow. They result from these directly and represent their adult form. Any other origin for the polynuclear neutrophiles has not been satisfactorily established. Ehrlich, it is true, has admitted that a certain number of the cells may develop in the circulation from the large mononuclear leucocytes with the so-called transition-forms as intermediate stages; but, as I have pointed out, there is a strong tendency at present to regard the transition-form as the terminal stage in the development of the large mononuclear leucocyte and to separate this group altogether from the granular varieties. There is an ontogenetic relationship between the two, but not a cytogenetic relation.

The polynuclear neutrophiles are the most common leucocytes of the blood and normally constitute from 60 to 70 per cent. of the total number. In young children they are relatively less numerous excepting during the first twenty-four hours of life, when they may number 73 per cent. But they rapidly diminish, so that values of from 20 to 40 per cent. may be regarded as normal during the first year. Low values continue practically to the twelfth year, though the numbers gradually rise. From the twelfth to the fourteenth year 60 per cent. may be regarded as an average; after that age the values given for the adult hold good.

A pathological increase in the number of the polynuclear neutrophiles constitutes the most common form of hyperleucocytosis (see page 86).

4. **The Polynuclear Oxyphilic or Eosinophilic Leucocytes** (Plate VI.).—In size and general appearance these cells resemble the polynuclear neutrophiles, and like these are capable of progressive locomotion and phagocytosis. The granules—the *a*-granulation of Ehrlich—however, are much larger and highly refractive, and possess a marked affinity for acid dyes, such as acid fuchsin and eosin. Hence the term *oxyphilic* or *eosinophilic* leucocytes. With neutral dyes or basic dyes they will not stain. The appearance of the individual granules varies somewhat in stained preparations. Some are round, others oval; some appear to stain throughout, others make the impression of little vesicles with a limiting membrane, which alone takes the dye, while the interior remains unstained. By means of MacCallum's method Barker[1] has shown that the granules contain iron. They are insoluble in ether and cannot be stained with osmic acid. They are therefore not composed of fat.

[1] Barker, Johns Hopkins Hosp. Bull., 1894, p. 93.

The protoplasm of the eosinophilic leucocytes is slightly basophilic. The nucleus is usually bilobed, sometimes trilobed, and in stained specimens it is quite common to find the individual lobes unconnected by threads of chromatin; often the two lobes are situated at opposite poles. As a rule the nucleus is less markedly basophilic than that of the neutrophilic variety.

The same degenerative changes which have been described in connection with the polynuclear neutrophiles may also be observed in the eosinophiles, and here, as there, one can at times note a material diminution in the number of the granules. I have never observed their entire absence, however, and it is noteworthy that in those cases of chronic leukæmia in which the neutrophilic granulation may disappear the eosinophilic variety remains.

Under normal conditions the percentage of the eosinophiles varies between 2 and 4, corresponding, according to Zappert, to minimum absolute values of 50–100 and maximum absolute values of 200–250, with 100–200 as an average.

While repeated attempts have been made to connect the eosinophilic leucocytes of the blood cytogenetically with the neutrophilic variety, there is insufficient evidence to support this view. On the other hand, there are strong reasons for believing with Ehrlich that, analogous to the neutrophilic variety, the polynuclear eosinophiles are normally formed in the bone-marrow, and here only from mononuclear eosinophilic cells—the *eosinophilic myelocytes*.

Unlike the neutrophilic granulation, the oxyphilic variety is represented in all vertebrate animals, and in birds may occur in the form of crystalloids.

5. **The Mast-cells (Polynuclear Basophilic Leucocytes)** (Plate VI.).—The mast-cells which are normally found in the blood are approximately of the same size as the polynuclear neutrophiles and eosinophiles. In myelogenous leukæmia, however, in which they are especially numerous, the size is more variable; on the one hand, they may only measure 3.5 μ in diameter, while on the other they may attain a dimension of 22 μ. The nucleus is polymorphous; but the tendency to form individual lobes is far less marked than in the corresponding eosinophilic and neutrophilic elements. Its affinity for basic dyes is quite feeble, so that it is often difficult in stained preparations to make out the boundary-line between nucleus and protoplasm. It is almost always excentrically located and usually has a fairly uniform diameter of 4 μ. In the smaller specimens the nucleus occupies almost the entire cell and is covered by a very thin layer of granules.

Embedded in the protoplasm lie granules of variable size—the γ-granulation of Ehrlich—some of which are fully as large as the eosinophilic granules, while others are much finer. They are characterized by their affinity for basic dyes and the fact that with certain ones they stain metachromatically, viz., in a color which is

different from that of the dye itself, which latter must be simple and not compound. Tissue elements which will stain in this manner are spoken of as chromotropic elements. Only a limited number of dyes have metachromatic properties. The most notable ones are the violet basic dyes hexamethyl-violet, cresyl-violet, thionin, neutral violet, and amethyst-violet; further, the blue dyes methylene-azure and toluidin-blue, and the red basic dyes pyronin, amidin-red, neutral red, and saffranin. With the latter group the mast-cell granules are colored yellow; with most of the violet dyes red, and with cresyl-violet R (extra) almost a pure brown. Methyl-green does not stain the mast-cell granules unless it is contaminated with methyl-violet, and for this reason the granules remain colorless in specimens that have been stained with Ehrlich's triacid stain.

The mast-cell granules are *absolutely* basophilic, viz., they can only be stained with basic dyes, and retain the basic dye on subsequent differentiation in acid media. They are capable, moreover, of taking up the basic dye from its acidified solutions, as in the case of Ehrlich's dahlia-acetic acid mixture (see page 136).

The granules of the common mast-cells of normal blood are quite resistant to water, while in myelogenous leukæmia cells are met with, the granules of which dissolve with great readiness (Michaelis). Their chemical nature is still a matter of dispute, but there is a tendency to associate the mast-cell with the formation of mucin. This, however, presupposes the identity of the blood mast-cell with the common mast-cell of connective tissue. In the past this has been tacitly assumed, but Pappenheim more especially has called attention to the fact that the hæmatogenic mast-cell differs from the histogenic form, and that the two probably represent different species.

The mast-cell apparently occurs in the blood of all vertebrate animals. In human blood their number rarely exceeds 0.5 per cent. Ewing states that he constantly failed to find mast-cells in the better class of healthy subjects, while in hospital and dispensary cases with minor ailments they appeared to be more numerous. My own observations do not bear this out; in my experience they are invariably present in health irrespective of the general nutrition of the individual.

The origin of the mast-cells of the blood has not been definitely ascertained. Ehrlich supposed that they originated from the connective-tissue cells as the result of hypernutrition, while Harris suggests that they may be derived from the large mononuclear leucocytes. According to Pappenheim, the mast-cell originates in the bone-marrow from a granular mononuclear type which corresponds to the eosinophilic and neutrophilic myelocytes.

6. **The Myelocytes.**—The myelocytes are mononuclear granular cells, which are *normally* not found in the circulation, but are encountered only in the bone-marrow.

Generally speaking, they represent the juvenile form of the polynuclear leucocytes of the blood, and we accordingly distinguish three varieties, viz., the neutrophilic, the eosinophilic, and the basophilic myelocytes. The last two named varieties, according to our present ideas, age directly into the corresponding polynuclear forms—*i. e.*, they become the common eosinophiles and the mast-cell. In the case of the neutrophilic variety it appears that two types exist, a smaller and a larger form, which Pappenheim[1] designates respectively as the trachychromatic and the amblychromatic form. These are ontogenetically derived, the first from the last, but only the trachychromatic variety ages into the common polynuclear neutrophiles of the circulating blood. The nucleus of the amblychromatic form as it matures likewise becomes polymorphous, but normally the cell remains an inhabitant of the bone-marrow even then.

As regards the origin of the myelocytes in turn, I incline toward Pappenheim's idea, according to which all three varieties result from the large lymphocytes through a process of heteroplastic differentiation.

a. The Neutrophilic Myelocytes.—These, as I have stated, are of two kinds. The one type, the *amblychromatic myelocyte* of Pappenheim, is a large cell provided with a relatively large centrally located round nucleus which stains but feebly with basic dyes. This is surrounded by a comparatively narrow zone of protoplasm which contains very fine neutrophilic granules. As the cell matures the nucleus becomes smaller and indented, so that forms result such as those pictured in Plate VI.; the position of the nucleus is then usually excentric. The protoplasm at the same time becomes relatively more abundant.

The second type, viz., the *trachychromatic myelocyte*, is a smaller cell, which is essentially characterized by the fact that its nucleus stains quite intensely with basic dyes. The protoplasm is faintly oxyphilic and the granulation rather coarser than in the amblychromatic variety. As this cell matures the protoplasm becomes relatively more abundant and the nucleus distinctly polymorphous; it then constitutes the common polynuclear neutrophile of the circulating blood.

Neutrophilic myelocytes undergoing mitosis are sometimes seen in the circulating blood in cases of myelogenous leukæmia; on the whole, however, they are rare, and it is more common to meet with cells in which the division of the nucleus has already taken place (Plate VI.).

Müller and Jolly have shown that the neutrophilic myelocytes of the circulating blood are capable of active locomotion.

b. The Eosinophilic Myelocytes.—In the more mature forms the color of the eosinophilic granulation on staining with

[1] A. Pappenheim, Virchow's Archiv, vols. clix. and clx.

eosin-methylene-blue mixtures is a pure eosin-red. The younger forms, however, present a purplish-violet color, and some granules may indeed be seen which are a pure blue (Plate VI.). This appearance is owing to the fact that the young eosinophilic granule is physically cyanophilic and chemically amphophilic, whereas the mature granule is physically erythrophilic, but chemically absolutely oxyphilic. This is well shown by staining such young cells with a mixture in which the basic dye is of a light color and the acid component dark, such as vesuvin on the one hand and water-blue on the other. The mature eosinophilic granules will then take on the blue color of the water-blue, while the young granules which stained blue with the eosin-methylene-blue mixture, and which we might accordingly have regarded as basophilic, are now likewise colored by the acid blue instead of the basic vesuvin, thus showing that they are in reality not basophilic, but amphophilic-cyanophilic.

The protoplasm of the eosinophilic myelocytes is basophilic.

The size of the cells is quite variable; some are considerably larger than the corresponding polynuclear form, while others are much smaller. The cyanophilic cells are, generally speaking, the largest.

According to the observations of Müller and Jolly, the eosinophilic myelocytes are capable of progressive locomotion.

c. The BASOPHILIC MYELOCYTES, like the eosinophilic and neutrophilic varieties, may be of variable size and are provided with a large centrically located nucleus, which is often distinguished only with difficulty from the surrounding protoplasm.

7. **Ehrlich's Neutrophilic Pseudolymphocytes.**—These bodies, according to Ehrlich, are produced by direct division of the polynuclear neutrophilic leucocytes. They are about as large as the small lymphocytes and provided with a single deeply staining nucleus, which is surrounded by a narrow zone of protoplasm containing neutrophilic granules. They differ from the small trachychromatic myelocytes essentially in their small size. They are rarely seen. Ehrlich states that he first observed them in a case of hemorrhagic smallpox, but that they also occur in recent pleural effusions. The cells no doubt are degeneration-forms and do not represent a separate species.

8. **Irritation Forms.**—These are mononuclear non-granular cells, the protoplasm of which is stained a rich brown by the triacid mixture. The nucleus is round, often excentrically located, and colored a bluish green. The smallest forms are somewhat larger than the lymphocytes. According to Türk, who first described these cells, they are met with under the same conditions as the myelocytes. Possibly they represent an early stage in the development of the nucleated red corpuscles.

Iodophilia.—On staining blood-smears of normal individuals with iodine (see page 138) the protoplasm of the leucocytes is colored a

bright yellow, while the nucleus is somewhat refractory and takes on a lighter tint. Under certain pathological conditions this staining quality is modified; cells are then seen in which reddish-brown granules appear in the protoplasm or it may occur that this presents a diffuse brownish color throughout. This intracellular reaction affects the polynuclear neutrophiles almost exclusively; the mononuclear elements *may*, however, also react, in which case one commonly sees large pale-brown granules arranged about the nucleus in a single row. In eosinophiles the reaction does not occur. The extent to which the leucocytes are involved is quite variable; in some cases a few cells only are affected, while in others one is scarcely able to find a normal cell in an entire preparation.

An extracellular reaction also occurs, but is of little clinical interest, as it is not infrequent even in health; it occurs in small roundish or oval masses, which are possibly true plaques, but which may also be small bits of protoplasm derived from leucocytes.

As to the nature of the substance which reacts with the iodine in the manner indicated, there is no uniformity of opinion. Ehrlich regards it as glycogen, and assumes that this is present normally in every cell in the form of a colorless compound, from which the free glycogen is under certain conditions split off, and can then be demonstrated as such. Czerny, on the other hand, looks upon the iodophilic substance as an antecedent of amyloid, while Goldberger and Weiss view it as peptone. Kaminer has shown that normal bone-marrow does not contain iodophilic leucocytes, but that they may here be found when they are present also in the blood. He concludes that the reaction is a degenerative phenomenon and not an evidence of regeneration.

The occurrence of the reaction in disease has been studied especially by Gabritschewsky, Czerny, Livierato, Kaminer, Cabot, and Locke. From these investigations it appears that septic conditions of all kinds furnish a positive reaction. Locke's list of diseases of this order includes general septicæmia, abscesses (excepting in the earliest stages), appendicitis accompanied by abscess formation, general peritonitis, empyema, pneumonia, pyonephrosis, salpingitis with severe inflammation or abscess formation, tonsillitis, gonorrhœal arthritis, and acute intestinal obstruction where the bowel has become gangrenous. Locke concludes that no septic condition of any severity can exist without a positive reaction. In puerperal sepsis also it is constant (Kaminer). In pneumonia with frank resolution it disappears in from twenty-four to forty-eight hours following crisis. In typhoid fever a positive reaction is not commonly obtained before the end of the second week, and it may indeed remain absent throughout the course of the disease. In the differential diagnosis between a serous and a purulent pleuritic effusion the absence of the reaction points to the former condition.

In contradistinction to chlorosis, pseudoleukæmia, and the common forms of secondary anæmia of moderate intensity, iodophilic leucocytes are found only in the severer forms of anæmia, such as pernicious anæmia, leukæmia (notably in acute cases), and the *severe* forms of secondary anæmia.

In animals the reaction can be produced artificially by infection with the streptococcus, the staphylococcus, the bacillus pyocyaneus, Löffler's bacillus, the anthrax bacillus, that of Friedländer, the bacillus coli communis, or the typhoid bacillus; as also by means of ricin, abrin, and the diphtheria toxin. Following the injection of oil of turpentine, croton oil, mustard oil, and silver nitrate, the reaction may occur even though bacterial infection has been avoided. In man it is also said to occur following narcosis.

LITERATURE.—Ehrlich, Zeit. f. klin. Med., 1882, vol. vi. Gabritschewsky, Arch. f. exp. Path. u. Pharmak., 1891, vol. xxviii. Czerny, Ibid., 1893, vol. xxxi. Goldberger u. Weiss, Wien. klin. Woch., 1897. Hofbauer, Centralbl. f. inn. Med., 1899. Livierato, Deutsch. Arch. f. klin. Med., 1894, vol. liii. Kaminer, Berl. klin. Woch., 1899, p. 119; and Deutsch. med. Woch., 1899, p. 206. Cabot and Locke, Jour. Med. Research, 1902, vol. vii. Locke, Boston Med. and Surg. Jour., 1902, p. 289.

Leucocytosis.

While the number of the red corpuscles is normally fairly constant, that of the leucocytes is subject to not inconsiderable variation. It is influenced by the age and sex of the individual, the process of digestion, menstruation, pregnancy, the blood-vessel from which the specimen of blood is taken, etc. Generally speaking, the number of the leucocytes varies between 3000 and 10,000, the exact number, *cæteris paribus*, depending upon the state of nutrition of the individual. In ill-nourished persons low values are the rule, while maximum numbers are generally associated with a state of exceptional vigor and good nutrition; 5000–7000 may be regarded as average values.

An increase in the number of leucocytes is met with under the most diverse conditions, both in health and disease. When transitory, it is commonly designated as *leucocytosis*. But, as Goldscheider has rightly suggested, it would be better to restrict this term to indicate the number of the leucocytes in a general way, and to speak of an increase as *hyperleucocytosis*, and of a decrease as *hypoleucocytosis*. For the latter condition the term *leucopenia* has also been suggested.

Ehrlich distinguishes between two forms of hyperleucocytosis, viz., an active and a passive form, the active form involving an increase of the polynuclear elements, while in the passive form the mononuclear cells are increased. This classification is based upon the assumption of absence of the power of locomotion on the part of the lymphocytes more especially, as contrasted with the polynuclear

leucocytes. In the light of recent investigations this distinction can, however, no longer be upheld, since we know that the lymphocytes are not only capable of changing their form, but, like the polynuclear elements, are also doubtless subject to the laws of chemotaxis.

As any variety of the leucocytes may be either increased or diminished, it is convenient for practical purposes to consider both possibilities in connection with each of the five normal types. We accordingly recognize:

1*a*. A polynuclear neutrophilic hyperleucocytosis.
1*b*. A polynuclear neutrophilic hypoleucocytosis.
2*a*. A polynuclear eosinophilic hyperleucocytosis.
2*b*. A polynuclear eosinophilic hypoleucocytosis.
3*a*. A mast-cell hyperleucocytosis.
3*b*. A mast-cell hypoleucocytosis.
4*a*. A large mononuclear hyperleucocytosis.
4*b*. A large mononuclear hypoleucocytosis.
5*a*. A lymphocytosis.
5*b*. A lymphopenia.

The term *myelæmia*, or, as I should suggest, *myelocytosis*, may be used to designate the appearance of myelocytes in the circulating blood, and in conformity with the three recognized forms we may speak of a neutrophilic, an eosinophilic, and a basophilic or mast-cell myelocytosis.

Until quite recently the general tendency in clinical laboratories has been to lay especial stress upon absolute numbers of leucocytes, and to neglect the relative values of the individual forms. This should not be, and I cannot insist too strongly upon the importance of the relative count, which in many respects is far greater than a knowledge of the total number. For this reason also I have chosen the consideration of the subject of hyperleucocytosis on the basis of the classification just outlined.

Polynuclear Neutrophilic Hyperleucocytosis.—This is the most common form of hyperleucocytosis, and, as the term indicates, principally affects the polynuclear neutrophiles. Exceptionally it may be associated with a polynuclear eosinophilia, as well as with a lymphocytosis; but as a general rule both eosinophiles and lymphocytes are diminished. This diminution is often not only relative, but absolute as well. In very marked cases of hyperleucocytosis of this type it is not uncommon to meet with a few myelocytes, which are then also of the neutrophilic variety; this is especially the case in children in whom the bone-marrow reacts more readily to stimulation. Eosinophilic myelocytes, on the other hand, are but rarely seen.

Clinically we must distinguish between an increase of the polynuclear neutrophiles which may occur in health and the common

hyperleucocytosis which is observed in disease. We may accordingly speak of a physiological and a pathological form.

Physiological Hyperleucocytosis.—A physiological increase in the number of the leucocytes is notably observed at birth, during the process of digestion, in pregnancy, in association with severe muscular exercise, following the use of cold baths, etc.

Leucocytosis of the Newborn.—According to the experience of most observers, the number of the leucocytes at birth varies between 10,000 and 23,000, of which over 70 per cent. are polynuclear neutrophiles. The number then falls and at the same time the lymphocytes increase. The curves of the two varieties cross between the sixth and the ninth day, and by the twelfth day the lymphocytes are in excess. From the end of the first month to the fourteenth year there are then a gradual increase of the neutrophiles and a decrease of the mononuclear elements (Carstanjen). During the first year the total number of the leucocytes varies between 10,900 and 12,900 (Gundobin).

Digestive Leucocytosis.—The increase in the number of the leucocytes which is observed during the process of digestion affects both the polynuclear elements and the lymphocytes, though especially the latter. The eosinophiles are relatively at least diminished (Rieder). The total increase rarely exceeds 3500 in normal adults, while in young children it may be much more marked. Schiff[1] thus cites an instance in which 19,500 leucocytes were counted one hour after birth, 27,625 after the first meal, and 36,000 after the fourth meal. It is especially pronounced after a preliminary period of fasting and following a meal rich in proteids. The maximum increase is usually observed between the third and fourth hour.

In cases in which a hyperleucocytosis exists from other causes, as in pregnancy, in inflammatory diseases, etc., digestive hyperleucocytosis does not occur. In a few isolated instances it has also been found absent in apparently normal individuals without assignable cause. Under pathological conditions its absence is not uncommon, even though hyperleucocytosis referable to other factors may not exist. This is notably the case in carcinoma of the stomach, and it was once thought that the absence of digestive hyperleucocytosis in doubtful cases could be interpreted as evidence in favor of its existence.[2] Generally speaking, this is true even now, and we may say that in about 90 per cent. of all cases of carcinoma of the stomach digestive hyperleucocytosis does not occur. The symptom, however, is not pathognomonic, as a number of instances of carcinoma have been reported in which there was a distinct increase, and as digestive leucocytosis *may* also be absent in other conditions. In anæmic

[1] Schiff, Zeit. f. Heilk., vol. xi. p. 30, and 1890, p. 1.

[2] Schneyer, "Das Verhalten d. Verdauungsleukocytose b. ulcus rotundum u. carcinoma ventriculi," Zeit. f. klin. Med., vol. xxvii. p. 249.

individuals, from whatever cause, especially large amounts of proteids are sometimes necessary to elicit an increase of the leucocytes (Müller[1]) and in some cases a subnormal number may even be encountered (Rieder[2]).

To study digestive hyperleucocytosis, it is well to proceed as follows:

a. The first blood-count should be made after the patient has fasted for about seventeen hours.

b. After this period he receives a test-meal consisting of from 200 to 1000 c.c. of milk, and one or two eggs, the amount varying with the condition of the patient.

c. Further blood-counts are made one, two, three, and four hours later.

d. The existence of a digestive hyperleucocytosis should only be regarded as proved if an increase of at least 1500 cells occurs, providing that maximal amounts of food have been taken. If smaller amounts have been given, an increase of 1000 cells is sufficient to establish its existence, provided that the same result is observed on repeated examination.

Leucocytosis of Pregnancy and Parturition.—The hyperleucocytosis which is observed in pregnancy is particularly marked during the last five months, and appears to occur quite constantly in primiparæ, while in multiparæ exceptions are common. In an analysis of 55 cases Hubbard and White[3] obtained positive results in 44—*i. e.*, in 80 per cent.—most marked and constant in young primiparæ. Rieder in an analysis of 31 cases noted a hyperleucocytosis in 20, all the negative cases being multiparæ. In a series of 17 multiparæ an increased number of leucocytes was noted in only 7. In Rieder's series the number of leucocytes varied between 10,000 and 16,000, with an average of 13,000. This represents the usual increase, but at times much larger numbers may be observed; Cabot thus reports 3 cases with a leucocytosis of from 25,000 to 37,000.

During actual labor there is an increase of the leucocytes over and above the numbers previously observed in pregnancy; 30,000 cells may then be noted. The highest numbers are met with in severe and protracted cases, especially after rupture of the waters. This form of hyperleucocytosis subsides after the expulsion of the child, and at the end of the first or second week normal values are again reached, though the gradual decline may be interrupted by a temporary increase now and then, referable to various minor disturbances during the puerperal state.

As in the case of the digestive leucocytosis, the hyperleucocytosis of pregnancy and the puerperal state is brought about by an increase

[1] R. Müller, Zeit. f. Heilk., 1890, p. 213.
[2] Rieder, Beit. z. Kenntniss. d. Leukocytose, 1892.
[3] Hubbard and White, Jour. of Exper. Med., 1898, p. 639.

PLATE VII.

The Blood of Pernicious Anæmia.

Note (*a*) the variations in the size and form of the red corpuscles; (*b*) the existence of polychromatophilic (1) *a* and granular *b* (2) degeneration in some of the red corpuscles; (*c*) the presence of nucleated red corpuscles, both of the normoblastic (3) and megaloblastic type (4); (*d*) the presence of free nuclei (5), derived from nucleated red corpuscles. (Bausch and Lomb, Eye-piece 1 inch, objective 1-12th.)

both of the polynuclear neutrophiles and the lymphocytes, while the eosinophiles remain passive.

Leucocytosis following Baths, Muscular Exercise, etc.—The increase of the leucocytes following cold baths may, according to Thayer, amount to nearly 300 per cent.[1] In 20 cases of typhoid fever he found 7724 leucocytes on an average before, and 13,170 after the usual Brand bath. In his own person, while in health, the leucocytes on one occasion numbered 3250 before the bath, while twenty minutes later they had increased to 12,500. Such an increase is, however, only observed after a bath of moderate duration, while a prolonged cold bath diminishes the number. Hot baths have exactly the opposite effect, viz., those of short duration produce a decrease, those of long duration an increase.

Violent muscular exercise, as well as massage, produces a temporary hyperleucocytosis.

Pathological Hyperleucocytosis.—1. *The Hyperleucocytosis of the Acute Infections.*—In the acute infectious diseases hyperleucocytosis referable to an increase of the polynuclear neutrophiles is the rule. It is thus seen in pneumonia, erysipelas, diphtheria, scarlatina, the various septic conditions, parotitis, acute articular rheumatism, etc. Typhoid fever and measles represent notable exceptions if we disregard the very earliest stage in the development of the disease, when an acute hyperleucocytosis may also be observed (see page 96).

Generally speaking, the increase in the number of the leucocytes in the acute infectious diseases is directly proportionate to the intensity of the infection and the power of resistance on the part of the individual. Where this is particularly feeble or the virulence of the infection is especially intense, an absolute increase of the total number of the leucocytes may not take place, although a relative increase of the polynuclear neutrophilic elements will probably always be observed. A recognition of this fact is of importance from the standpoint both of prognosis and of diagnosis, and serves to illustrate the special value of the *differential* count.

In *pneumonia* the increase in the number of the leucocytes is usually marked. On an average it amounts to about 24,000 cells above the normal (Cabot). The hyperleucocytosis sets in quite early —within a few hours following the initial chill—and persists until the time of the crisis, when it rapidly disappears; the decrease may indeed precede the critical fall of the temperature. When the disease terminates by lysis the return to the normal is more gradual. A pseudocrisis is not accompanied by a fall in the number of the leucocytes. When resolution is delayed or complications occur, the hyperleucocytosis persists. Excepting very mild cases the prognosis is especially grave when a well-marked hyperleucocytosis does not

[1] Thayer, Johns Hopkins Hosp. Bull., April, 1893.

occur. Sears and Larrabee[1] found the mortality much greater when the leucocytes numbered less than 10,000 than when they were more numerous; and according to Löper, a progressive increase of the neutrophiles beyond 90 to 95 per cent. may be regarded as an unfavorable symptom irrespective of their total number. Absence of hyperleucocytosis, excepting in very mild cases, will usually warrant a fatal prognosis; exceptions, however, occur, and it is well in any case to base prognostic conclusions not upon a single count, but upon the result of repeated examinations. It is not uncommon to meet with considerable fluctuations in the leucocyte count in the course of the disease. Associated with the increase of the polynuclear neutrophiles in pneumonia there is a relative diminution of the lymphocytes. The eosinophiles at the height of the disease are greatly diminished; they may indeed be absent. Their return may occur before the beginning of the crisis and may be viewed as a favorable symptom.

In *bronchopneumonia* the total increase of the leucocytes is not so extensive as in the acute croupous form.

In *erysipelas*, as in pneumonia, the hyperleucocytosis is generally proportionate to the intensity of the morbid process and also terminates by crisis. The increase of the leucocytes beyond normal may amount to 15,000 (Rieder); in many cases, however, the total number does not rise much beyond the upper limit of the normal. At the height of the disease the eosinophiles are much diminished or absent.

In *diphtheria* a well-marked increase is the rule. Generally the count does not exceed 25,000 to 30,000, but in fatal cases it is common to meet with larger numbers. Ewing[2] speaks of one case with lymphocytosis in which the count was 72,000, and cites a peculiar instance reported by Felsenthal[3] marked by hemorrhagic eruption in which 148,000 were counted. As Ewing suggests, this was probably an agonal hyperleucocytosis. As a rule, from 25,000 to 50,000 cells are met with in fatal cases. In children the general increase of the leucocytes is frequently associated with a relative lymphocytosis. The eosinophiles are diminished in number and may indeed be absent. It is interesting to note that excepting a temporary diminution immediately following the injection, the leucocytosis is in no wise influenced by the antitoxin treatment. Besredka,[4] however, states that the grade of the polynuclear neutrophilic hyperleucocytosis after the administration of the serum indicates the prognosis. Thus, if one or two days after the injection the percentage of the neutrophiles is 60 or more, the prognosis is good; with a higher tempera-

[1] Sears and Larrabee, Med. and Surg. Rep. of the Boston City Hosp., 1901, 12th series, Dec. 1st.

[2] Ewing, On the Blood, loc. cit.

[3] Felsenthal, Arch. f. Kinderheilk., vol. xv. p. 78.

[4] Besredka, Annal. de l'Inst. Pasteur, 1898, vol. xii. 5, p. 305.

ture and 50 per cent. it is bad, and with a lower percentage than 60 the disease is fatal. The exanthem which occasionally follows the injection of antidiphtheritic serum is accompanied by a polynuclear neutrophilic hyperleucocytosis.

In *tonsillitis* there is an increase of the leucocytes of approximately the same intensity as in diphtheria, with a similar diminution in the number of the eosinophiles.

In *septic conditions*, in general, hyperleucocytosis is of constant occurrence at some stage of the disease, unless the infection is very mild or very severe. Even in those cases in which the absolute increase of the leucocytes is not especially marked, or, as in certain very virulent cases is absent altogether, *the neutrophiles are relatively increased and the eosinophiles coincidently very much diminished or absent altogether.* Osteomyelitis forms the only apparent exception to this rule in so far as the eosinophiles are concerned.

Especially important is the study of the leucocytosis in *appendicitis.* I here quote from Bloodgood's paper on the value of blood examination in surgical diagnosis :[1]

Observed within forty-eight hours the number of white blood-cells is in the majority of instances of great value in pointing to the extent of the inflammatory condition of and about the appendix. Cases of recurrent appendicitis or of appendicitis suffering from the first attack, first observed practically at the end of the attack when the clinical symptoms are subsiding, rarely show an increase in the white cells. In a few instances, first observed within forty-eight hours after the beginning of the attack, but when the symptoms are subsiding, there have been a few leucocyte-counts of 15,000, which have fallen rapidly within a few hours to 10,000 and 7000. In the cases admitted within forty-eight hours with acute symptoms, if on account of the clinical picture operation has been delayed, a falling leucocytosis has always been observed. These patients have recovered, and at a later operation the appendix was found to be the seat of a diffuse inflammation, but there was no evidence of pus outside the appendix. In one case admitted sixteen hours after the beginning of the attack the leucocytes fell in ten hours from 17,000 to 13,000, and in twenty-four hours to 11,000, associated with disappearance of the symptoms. With one exception, the highest first leucocyte-count in this group has been 17,000, falling in a few hours to 12,000, 9000, or even lower. A patient admitted twenty hours after the beginning of the acute attack had a leucocytosis of 22,000; the clinical symptoms, however, were not very marked. The patient was observed eight hours; during this period the leucocytes fell to 16,000 and the local symptoms practically disappeared. Within the succeeding twenty-four hours the leucocytes were 11,000,

[1] Bloodgood, "Blood Examination as an Aid to Surgical Diagnosis," Am. Med., 1901, p. 306.

then 8000, 7000, and 6000. Although this patient with a leucocytosis of 22,000 at the end of twenty hours, recovered, and there is every reason to believe that the inflammatory condition about the appendix subsided, nevertheless it is an exception to the general rule, and it would be safer, I believe, to operate in those cases of acute appendicitis observed within the first forty-eight hours with a leucocytosis of 20,000.

In acute diffuse appendicitis with operation and recovery the highest count observed was 25,000 thirty-six hours after the beginning of the attack. At operation in this case intense inflammation and a large amount of exudate were found about the appendix.

In gangrenous appendicitis with operation and recovery the leucocytosis is higher (25,000–35,000) and rises more rapidly. As Bloodgood says, the study of the leucocytosis is here of the greatest importance in the early recognition of a grave inflammatory condition of the appendix, which without doubt would lead to general peritonitis and death unless early operation be instituted.

A very high leucocytosis within forty-eight hours after the beginning of the attack is suggestive, but not at all positive, of beginning *peritonitis*. The leucocyte-count, however, does not seem to help in such cases with regard to prognosis. After the second day in cases in which the peritonitis has been present longer Bloodgood never has observed recovery with a low leucocyte-count. If the leucocytosis remains still high at this period, however, the prognosis seems better for ultimate recovery after operation.

In intestinal obstruction an increase of the leucocytes associated even with very slight symptoms is of the highest importance in the early recognition of the lesion. Bloodgood states that in a large group of cases the leucocyte-count may rise to 20,000 within twelve hours after the beginning of the obstruction. Within the first twelve to twenty-four hours a few observations would demonstrate that if the leucocyte-count rise above 25,000 or 30,000, the probabilities are that one will find gangrene of the obstructed loops or beginning peritonitis. If observed on the second or third day after the beginning of the symptoms, it is difficult to make a differential diagnosis with regard to gangrene or peritonitis. After the third day, in cases in which there is no gangrene, and no peritonitis, or in which the auto-intoxication is not yet very grave, the leucocytes still remain high—15,000–23,000—according to the degree of obstruction : complete, higher ; partial, lower. In the presence of gangrene-peritonitis or grave auto-infection, the leucocytes begin to fall. If the patient is admitted after the third or fourth day, with a history of intestinal obstruction, and still has a high leucocyte-count, the prognosis is good for operation. If the count is low, and especially if it is below 10,000, the probabilities are that on operation extensive gangrene-peritonitis will be found; or the

patient will be so depressed by auto-intoxication that reaction does not follow relief of the obstruction.

In *scarlatina* hyperleucocytosis is a constant feature of the disease.[1] It usually begins two or three days before the appearance of the rash; sometimes even as early as the sixth day. The acme is reached on the second or third day; on the fourth medium values are found. Then the decrease usually begins, although this is sometimes delayed until the eighth or ninth day; normal values are not reached until the end of the second or the beginning of the third week. In light cases the leucocytosis amounts to from 10,000 to 20,000 cells, in cases of moderate severity 20,000 to 30,000 are average figures, while in fatal cases 40,000 are common values. The hyperleucocytosis is scarcely influenced by the height of the temperature, the angina, the rash, desquamation, or complications, excepting that in the latter case its duration is influenced by the nature of the pathological process. The hyperleucocytosis is due to a large increase of the polynuclear neutrophiles, which may represent 94 per cent. of all leucocytes. The lymphocytes are proportionately diminished unless glandular complications occur, when they may reach maximum normal values. The eosinophiles in light and moderately severe cases are at first normal or subnormal, they then gradually increase and reach maximum values (8–15 per cent.) in the second or third week, after which they return to normal. In severe cases they diminish to zero (see also page 101).

In *acute articular rheumatism* the degree of hyperleucocytosis is proportionate to the severity of the attack. In McCrae's[2] analysis of 83 cases the average count was 11,776; in 29 it was below 10,000. Taking the average of the remaining 54 cases we have 14,260. In 17 the count was over 15,000 and in 4 over 20,000; the highest figure was 38,000. It is noteworthy that hyperleucocytosis was noted in all cases of complicating pericarditis in which a count was made, but that normal values were obtained in many cases of undoubted endocarditis. In pericarditis 15,000 to 19,000 were average values; 35,000 was the highest count noted. Generally speaking, when the number of leucocytes in acute articular rheumatism rises to 20,000 or higher, pericarditis or pneumonia may be suspected (Türk, Ewing). When the total increase of the leucocytes is only slight, the percentage values are not especially disturbed, but with a marked hyperleucocytosis the polynuclear neutrophiles are materially increased. The eosinophiles are commonly absent in the early stages of the disease, while later they are always present in moderate numbers, and after defervescence they are usually increased.

[1] Van der Berg, Arch. f. Kinderheilk., vol. xxv. p. 321. Mackie, Lancet, Aug. 24, 1901. Reckzeh, Zeit. f. klin. Med., 1902, vol. xliv. p. 201 (full literature).

[2] McCrae, Jour. Am. Med. Assoc., 1903, vol. xl. p. 210.

In *tubercular disease* hyperleucocytosis is observed only when secondary infection with pus organisms has taken place, while in pure cases the number remains normal. As the conditions for a secondary infection are more favorable in some parts of the body than in others, such as the lungs and kidneys, hyperleucocytosis is commonly present when these parts are involved. In the third stage of pulmonary tuberculosis there is usually a leucocytosis of from 15,000 to 20,000, which is referable to a well-marked increase of the polynuclear neutrophiles, while the eosinophiles are diminished. In the second stage, owing to a concentration of the blood no doubt, values ranging between 8000 and 10,000 are common, while in the first stage normal values are found.[1] In tubercular peritonitis the leucocytosis is variable. In 36 cases of 46 analyzed by Shattuck the number was below 10,000; where it is higher pus may or may not be present. In tubercular meningitis there is as a rule no increase in the number of the leucocytes; but in a few instances counts between 14,000 and 34,000 have been reported. When hyperleucocytosis does occur, it may be due to a complicating terminal pneumonia.

In non-tubercular *meningitis* hyperleucocytosis is well marked; it appears early in the disease and persists until death.

In *smallpox* a hyperleucocytosis is observed only in the severer cases and when pustulation takes place. In the milder forms no increase occurs.

In *Malta fever* a marked increase of the polynuclear neutrophiles may occur just before the onset of the fever; later there is absence of hyperleucocytosis (Bruce). In a case observed in the United States by Musser and Sailer 11,564 leucocytes were counted, all varieties being present in normal proportion.[2]

In *bubonic plague* a moderate increase of the leucocytes is the rule; a few instances have been reported in which over 100,000 cells were counted, the increase being largely due to the neutrophiles.

In uncomplicated cases of *typhoid fever*, as I have indicated, the leucocytes are diminished except during the first days, when there may be a leucocytosis of 3000–5000 beyond the normal, referable to an increase of polynuclear neutrophiles. After this, however, the leucocytes diminish and a relative lymphocytosis gradually comes to the foreground (see especially page 96).

In *uncomplicated measles* there is in the beginning a moderate relative increase of the polynuclear neutrophiles, 76–82 per cent.; but this is not associated with an absolute increase of the leucocytes, but with a decrease. Later there is a relative decrease of the neutrophiles to 50–60 per cent., while the absolute number is increased.

According to Wilson and Chowning, a hyperleucocytosis of about

[1] Appelbaum, loc. cit., p. 61.
[2] Musser and Sailer, Phila. Med. Jour., 1898, p. 1408, and 1899, p. 89.

12,000 is usual in cases of the so-called *spotted fever* of the Rocky Mountains.[1]

2. *Anæmic Hyperleucocytosis.*—Hyperleucocytosis referable to an increase of the polynuclear neutrophiles is observed in various forms of acute and chronic anæmia. It is especially marked after hemorrhages, when the number of leucocytes may increase to 30,000 and even more. Generally speaking, the degree of increase is here proportionate to the amount of blood lost and the recuperative power of the individual. In the human being Rieder noted a leucocytosis of 15,000 after a pulmonary hemorrhage; 32,600 after a hemorrhage due to uteric cancer, and 26,500 after a hemorrhage referable to gastric ulcer.

If we except the myelogenous type of leukæmia, in which an absolute increase of the polynuclear neutrophiles is associated with a relative decrease, hyperleucocytosis is not met with in uncomplicated cases of the primary anæmias. In the secondary forms, however, it is quite common, though usually of moderate degree.

3. *Cachectic Hyperleucocytosis.*—A cachectic hyperleucocytosis has been described in connection with malignant disease, phthisis, etc. Ewing states that in the majority of cases of tertiary syphilis, tuberculosis, and nephritis, in a large proportion of carcinoma cases and in a rather smaller proportion of sarcomas the cachexia is unaccompanied by hyperleucocytosis unless there is distinct local inflammation, necrosis, or hemorrhage. He suggests that the existence of a marked hyperleucocytosis in the course of a cachexia should lead to a search for one of these complications.

4. *Ante-mortem Hyperleucocytosis.*—An ante-mortem hyperleucocytosis has been described by Litten[2] and others in moribund individuals, in which no increase in the leucocytes had previously occurred.

5. *Hyperleucocytosis referable to Drugs.*—Hyperleucocytosis referable to an increase of the polynuclear neutrophiles has been observed in cases of poisoning with potassium chlorate, arsenious hydride, and illuminating gas. It follows the administration of atropine, quinine, the salicylates, thyroid extract, tuberculin, and the infusion of salt solution. It is noted after prolonged anæsthesia with chloroform and ether, when an increase of 5000–10,000 cells is quite common. This increase occurs after from six to forty-eight hours following the operation, and persists for only a few hours. A post-operative increase of 10,000 or more beyond the normal value of the individual and sustained for more than a few hours, should be looked upon with suspicion.[3]

6. *Hyperleucocytosis of Thermic Fever.*—In thermic fever a high

[1] Wilson and Chowning, Jour. Am. Med. Assoc., July 19, 1902, p. 131.
[2] Litten, Berl. klin. Woch., 1877, No. 51.
[3] Da Costa and Kalteyer, Am. Med., 1901, p. 306.

leucocyte count is apparently the rule, but there is considerable irregularity in the time and duration of the rise. Lewis and Packard[1] report that in some of their cases a leucocytosis of from 12,000 to 13,000 was noted on admission. In most of the cases in which there was a primary rise this was followed by a fall and then a second increase in their number.

In addition to these various forms of hyperleucocytosis an increase of the neutrophiles is further observed under conditions which do not as yet permit of an appropriate classification; in some cases no doubt the hyperleucocytosis is of toxic origin; in others it may be referable to an abnormal distribution of the cells; in still others to a coexistent anæmia, etc. Such conditions are rickets, gout, acute yellow atrophy, advanced hepatic cirrhosis (especially when associated with jaundice), acute gastro-intestinal disorders, acute and chronic nephritis, hydronephrosis, etc.

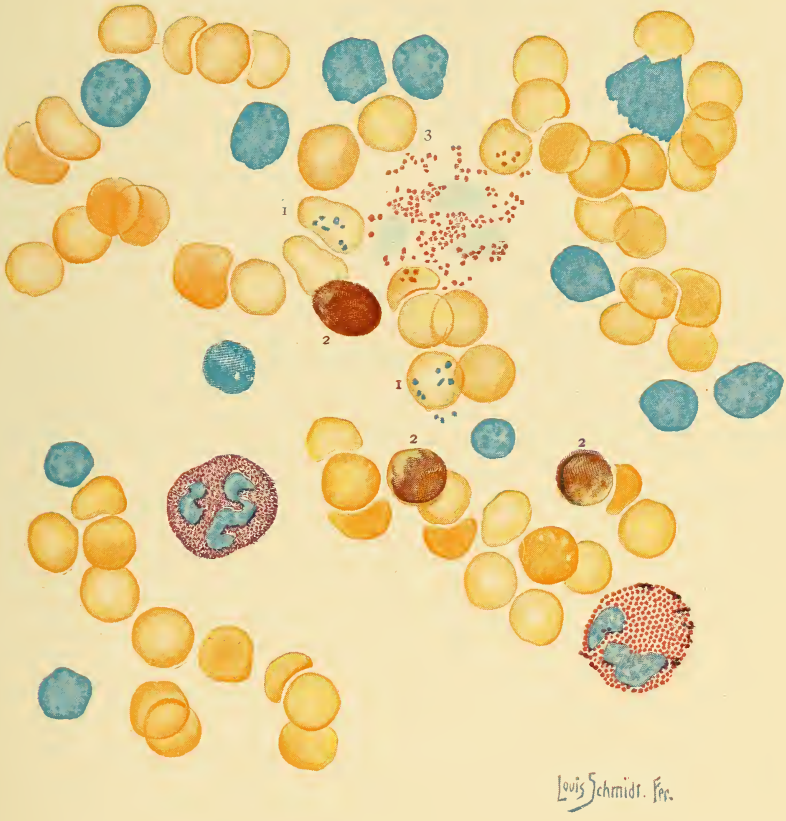
Polynuclear Neutrophilic Hypoleucocytosis (Leukopenia).— A diminution in the total number of the leucocytes is observed in only a comparatively small number of diseases, and is practically always referable to a decrease in the number of the polynuclear neutrophiles. It is notably observed in typhoid fever, measles, influenza, in certain anæmic conditions, etc.

In *typhoid fever*[2] hypoleucocytosis is so constantly seen that we can formulate the general rule that *whenever an increase in the number of the leucocytes is observed in a case of suspected typhoid fever it is more than probable that some complication exists or that the diagnosis is wrong.* Exceptions to this rule are rare. In the very earliest days of the disease, possibly owing to a concentration of the blood, the result of starvation and diarrhœa, higher counts are sometimes observed, but as the disease progresses the number soon diminishes, and in the later stages of the disease is practically always markedly below the normal. Not uncommonly they are less than 2000, and in some instances the number may indeed fall below 1000. The qualitative changes are especially important; and according to Nägeli, characteristic of the different stages of the disease. At first, while the temperature is steadily rising there is a neutrophilic hyperleucocytosis of moderate degree; this is associated with a moderate decrease of the lymphocytes, while the eosinophiles disappear. Then the neutrophiles diminish and the period of the hypoleucocytosis properly speaking commences. During this stage, viz., the stage of continued fever, the neutrophiles usually number from 3000 to 4000, as compared with 5000 to 6000 during the second half of the first

[1] Lewis and Packard, Trans. Assoc. Am. Phys., 1902, p. 409.

[2] Nägeli, Deutsch. Archiv, lxvii., Parts 3 and 4. Kölner, Ibid., lx. p. 221. Thayer, Johns Hopkins Hosp. Bull., 1901, vol. iii. p. 500; and Studies in Typhoid Fever, Johns Hopkins Press, 1901, p. 487.

PLATE VIII.



The Blood of Lymphatic Leukæmia.

Note the large increase of the lymphocytes. Two of the red corpuscles are undergoing granular degeneration (1); in a few others polychromatophilia (2) can be discerned; at 3 an eosinophilic leucocyte is seen with scattered grounds. (Bausch and Lomb, Eye-piece 1 inch, objective 1-12th.)

week. The lymphocytes are now also diminished, but tend to rise toward the end of this period; the eosinophiles are absent. During the third stage (remission) the neutrophiles decrease still further—1500–2500—while the lymphocytes increase and a few eosinophiles appear. In the fourth stage (defervescence) the neutrophiles reach their minimum, 900 in severe cases, while the lymphocytes are relatively much increased and the eosinophiles gradually return to normal. The reascent of the neutrophiles then occurs very slowly, while coincidently there is a lymphocytosis which is especially marked in children and continues far into convalescence. Normal values are sometimes not reached until after a couple of months.

In the event of a relapse occurring during an afebrile period there is a distinct neutrophilic hyperleucocytosis, the actual number depending upon the preceding counts, to which from 3500 to 5000 neutrophiles are added; at the same time the eosinophiles disappear. Should a relapse occur in the third stage of the disease, then the eosinophiles, which have just begun to reappear, disappear abruptly.

Favorable indications in cases of typhoid fever are an increase of the eosinophiles at the height of the disease; reappearance of the eosinophiles, indicating arrival at the third or fourth stage; an increase of the lymphocytes, which appears to begin only at a time when the severest part of the intoxication is over; not too great a decrease of the neutrophiles in the absence of complications. Unfavorable indications are: a marked decrease of all leucocytes, and especially of the lymphocytes; absence of hyperleucocytosis and a farther decrease of the neutrophiles in the event of complications, which *per se* would call forth a hyperleucocytosis.

In the event of complications the total number of the leucocytes frequently does not exceed the upper limit of the normal; but in such cases a differential count will reveal a relative increase of the neutrophiles.

In cases of perforation there is frequently an increase in the total number of the leucocytes, which may, however, be quite transitory and escape observation unless an early examination is made and previous counts are available; for later, when peritonitis is general, the leucocytes are usually found diminished; in some instances, however, there is no increase at the onset.

In one of Cabot's cases the count before operation was 8300, and immediately afterward 24,000. Finney reports a case with 6500 before and 10,600 after. In one of Cushing's cases there was an early recognized hyperleucocytosis which appeared before any sign of general peritonitis had developed; 8400 before and 16,000 after. In this patient it was interesting to note that following the operation the leucocytes fell to 4000; but immediately following the development of obstruction, due to kinking of the bowel, the leucocytes increased to 13,000 and later to 20,000, to

fall again following the removal of the obstruction. In a second case operated by Cushing there was a persisting hyperleucocytosis, associated with abdominal pain and tenderness, at one time reaching 15,200. Upon the development of general peritonitis the count showed only 4300. Cabot remarks, "steadily increasing leucocytosis is always a bad sign, and should never be disregarded, even when other bad symptoms are absent," to which Cushing adds, "a decreasing leucocytosis may be a much worse sign" (Finney[1]).

Measles[2] is the second notable exception to the general rule that the acute infections are associated with a polynuclear neutrophilic hyperleucocytosis. But it is interesting to note that here also the hypoleucocytosis is preceded by a pre-eruptive *hyperleucocytosis*, which commences at the beginning of the period of incubation, then increases rapidly and reaches its maximum about the sixth day before the appearance of the eruption. After this it diminishes, and at the appearance of the exanthem and during its course the occurrence of an increased number of leucocytes indicates some complication. The hypoleucocytosis affects the polynuclear neutrophiles both absolutely and relatively, while the lymphocytes are relatively at least increased. The eosinophiles disappear. The hypoleucocytosis generally reaches its maximum on the second day of the eruptive stage, when the number of leucocytes is reduced to about one-half. After this they increase again more or less rapidly and reach the normal from one to five days after the disappearance of the rash, unless some complication should supervene. Numerous eosinophiles then appear together with an absolute and relative increase of the polynuclear neutrophiles.[3]

Urticaria, syphilitic roseola, scarlatina, and the exanthem which may follow antitoxin treatment are not associated with hypoleucocytosis.

In uncomplicated cases of *tuberculosis* there is usually no increase of the leucocytes; when it does occur it is generally referable to suppurating cavities, recent hemorrhages, and resulting anæmia, or to advancing pneumonia. The increase which occurs under such conditions is moderate and does not often exceed 15,000 cells. Ewing states that he has seen both lungs consolidated and riddled with small cavities in a case lasting but five weeks, and yet the leucocytes were never found above 12,000. He suggests that the absence of leucocytosis in such cases of acute phthisis which resemble pneumonia may often be of value in diagnosis. Unfortunately there is no large series of examinations available from which to decide the relative value of the morphological examinations of the blood in the differential diagnosis between acute miliary tuberculosis and typhoid

[1] J. M. T. Finney, Surgical Treatment of Perforating Typhoid Ulcer. Studies in Typhoid Fever. Johns Hopkins Press, 1901, p. 170.

[2] Reckzeh, Zeit. f. klin. Med., vol. xlv. p. 107 (full literature).

[3] Renaud, Thèse de Lausanne, 1900.

fever. According to Cabot and Warthin, a subnormal number of leucocytes may also be observed in acute miliary tuberculosis, while Kölner[1] thinks the leucocyte count important in distinguishing between the two diseases.

In uncomplicated cases of *influenza* the total number of the leucocytes is commonly diminished; it may, however, be normal. In my experience there is usually a slight relative increase of the neutrophiles with diminution of the eosinophiles. When an absolute increase of the leucocytes occurs, some complication probably always exists.

In severe cases of *anæmia* the occurrence of hypoleucocytosis is always a grave symptom, as it indicates an inability on the part of the bone-marrow to produce a sufficient number of blood-corpuscles. Ehrlich supposes that in such cases the fatty marrow of the long bones is not transformed into red marrow, and he cites two cases in which the correctness of this supposition was demonstrated at the post-mortem table. A hypoleucocytosis of this order was observed by Decastello and Hofbauer[2] in five cases of pernicious anæmia, in four of chlorosis, in two of post-hemorrhagic anæmia, in two of liver abscess, one of phthisis florida, one of sepsis with severe anæmia, in three severe anæmias of unknown origin, in two cases of pseudo-leukæmia, and two of splenic anæmia.

In splenic anæmia[3] hypoleucocytosis appears to be a feature of the disease at some period in its course and is at times most marked. Osler mentions a case of Vickery's in which only 650 to 700 leucocytes were counted pro cbmm., and one of Peabody's with 800 cells. The average count in the series collected by Osler was 3850. Immediately after a profuse hemorrhage or in a *terminal affair* there may be a hyperleucocytosis.

In pernicious anæmia hypoleucocytosis is also the rule, and at times remarkably low counts are obtained. Strauss and Rohnstein[4] cite two cases with 400 and 328 cells, respectively. As a rule, however, the diminution is more moderate, and the general average not much below the minimum normal.

While the hypoleucocytosis in the diseases mentioned is rarely extreme, most extraordinary instances of leukopenia are at times encountered. Ehrlich[5] thus cites the case of a well-built young man in whom brief epileptiform seizures occurred, and in one of which the patient died. The post-mortem examination was entirely negative. During the three days of observation preceding death two examinations of the blood were made. On the first day not a single leucocyte could be demonstrated in ten blood-films, and on the second day but one was found in the same number of specimens.

[1] Loc. cit., p. 96.
[2] Decastello and Hofbauer, Zeit. f. klin. Med., vol. xxxix. p. 488.
[3] Osler, Am. Jour. Med. Sci., 1902, vol. cxxiv. p. 751.
[4] Strauss u. Rohnstein, loc. cit.
[5] Ehrlich, Die Anæmie, loc cit.

L of C.

Of *drugs*, atropine, camphoric acid, tannic acid, picrotoxin, agaricin, menthol, sulphonal, and several other antihydrotics, cause a marked decrease of the leucocytes.[1]

Polynuclear Eosinophilic Hyperleucocytosis (Eosinophilia).—A *physiological* increase of the eosinophiles beyond the maximum observed in adults is seen in young children. According to Zappert, the relative numbers may here vary between 0.67 and 11 per cent., and Müller and Rieder even speak of 21 per cent. In older children, however, normal adult values prevail, and it is then legitimate to consider an increase beyond these figures as abnormal.

It is stated by some that there is a physiological increase of the eosinophiles during the menstrual period and following coitus. This is inconstant, however, and rarely marked.

Eosinophilia is thus essentially a pathological phenomenon. It occurs under the most diverse conditions, as in the myelogenous type of leukæmia, in bronchial asthma, in various skin affections, the helminthiases, gonorrhœa, osteomyelitis, following the injection of tuberculin, etc.

In *myelogenous leukæmia* (Ehrlich) an absolute increase in the number of eosinophiles is one of the most constant symptoms of the disease. Ehrlich indeed has taught that this increase occurs in all cases and must be demonstrable to warrant the diagnosis. In view of recent advances in our knowledge of the pathology of the disease, however, this idea can no longer be upheld, as it has been shown that all forms of leukæmia are or at least may be of myelogenous origin.[2] Cases have been recorded in which the blood picture was essentially that of the orthodox lymphatic variety, but in which post-mortem examination showed a total absence of involvement of the lymph-glands, while the bone-marrow was extensively diseased. In these cases there was no increase in the total number of the eosinophiles. But it seems that even in those cases in which the blood picture is essentially that of a myelæmia the usual increase in the number of the eosinophiles may be lacking. I have thus reported an instance in which these cells were not only not increased, but were practically absent.[3] Such cases, however, are exceedingly rare, and it may still be regarded as the rule that in those cases of leukæmia in which extensive myelocytosis exists the eosinophiles are absolutely if not relatively increased. With septic complications occurring in the course of the leukæmias the eosinophilic leucocytes are materially diminished, and in some cases they may be absent altogether. Exceptions, however, occur, and Ehrlich cites a case in which the absolute number of eosinophiles was still between 1400

[1] Bohland, Centralbl. f. inn. Med., 1899, No. 15.
[2] Pappenheim, Zeit. f. klin. Med., vol. xlvii. p. 216.
[3] C. E. Simon, Am. Jour. Med. Sci., June, 1903.

and 1500 pro cbmm., although the percentage had diminished from 3.5 to 0.43.

In *bronchial asthma* an increase of the eosinophiles is observed quite constantly about the time of the paroxysm, and may amount to from 10 to 53.6 per cent.[1] Its occurrence is of value in differential diagnosis as renal and cardiac asthma are not associated with eosinophilia. Between attacks normal numbers are found (v. Noorden, Swerschewski).

In *scarlatina*[2] an increased number of eosinophiles is quite constantly observed at some time in the course of the disease. As the result of an analysis of 167 cases Bowie finds that at the onset of the fever they are diminished. In simple favorable cases they then increase rapidly until the height of the disease is passed, when they diminish again, and finally reach the normal some time after the general hyperleucocytosis has disappeared, viz., when the poison has all been eliminated. The more severe the case the longer are the eosinophiles subnormal before they rise again; in fatal cases they never rise, but rapidly decrease to zero. Bowie thinks that the curve of the eosinophiles is of value from a prognostic standpoint. If they are normal or subnormal after the first day or two, the case will in all probability be a severe one. In Reckzeh's series the highest percentage was 12.5, and the largest total number 1350.

In *measles* an increase of the eosinophiles does not occur.

In many *skin diseases* eosinophilia may also occur, as in pemphigus, prurigo, psoriasis, in Dühring's hydroa gravis, in urticaria, chronic eczema, etc. Generally speaking, the degree of increase in skin diseases is proportionate to the amount of tissue involved. In urticaria and pemphigus more particularly the increase may be very marked; in one case of urticaria 60 per cent. were noted, and in a case of pemphigus the absolute number of eosinophiles was 4800 (as compared with 250, the highest normal value). In some cases of leprosy percentages varying between 8.48 and 61 have been obtained, while in others, notably in the nervous form, normal values are found. In a case of epidermolysis bullosa hereditaria Brown met with 9.7 per cent. of eosinophiles; in one of severe chronic eczema with 24 per cent.

Of special interest is the increase of the eosinophiles in the *helminthiases*. It may occur in connection with all intestinal parasites, including the amœba. According to Leichtenstern,[3] it is most pronounced in those cases in which Charcot-Leyden crystals are numerous in the feces. The greatest increase has been observed in ankylostomiasis, where 72 per cent. were counted in one case. As a general rule,

[1] Billings, N. Y. Med. Jour., vol. lxv. p. 691.

[2] Zappert, Zeit. f. klin. Med., 1893, p. 292. Reckzeh, Ibid., vol. xlv. (literature). Bowie, Jour. Path. u. Bact., 1902, vol. viii. p. 82.

[3] Leichtenstern, Wien. klin. Rundschau, 1898; and Deutsch. med. Woch., 1887–88.

however, the eosinophilia is not so extensive. Herrick observed one case in which the cells numbered 26 per cent., and Kieffer gives 3–30 per cent. as usual values. In the presence of oxyurides Bückler[1] found 16 per cent.; 19 per cent. were counted in association with ascarides, and Leichtenstern reports one case of Tænia mediocanellata with 34 per cent. It is to be noted, however, that eosinophilia is not a constant feature in infections with the common tæniæ, oxyuris, and ascaris, and that the number of eosinophiles may not exceed minimum normal values. In cases of infection with the bothriocephalus eosinophilia does not occur (Schaumann). In a fatal infection with Balantidium coli Strong and Musgrave[2] observed a relative increase, and it appears that in amœbic colitis also a moderate eosinophilia is not uncommon.[3]

As Brown[4] has shown, a remarkable increase of the eosinophiles occurs in *trichinosis* during the acute stage. In his first 4 cases with a total leucocyte count of 35,000, 13,000, 17,000, and 18,000 the percentage of eosinophiles was 68.2, 42.8, 49, and 48, respectively. Kerr noted even a higher percentage in one case, viz., 86.6. Similar results have been obtained by Thayer,[5] Cabot,[6] Gwyn,[7] Blumer-Neumann,[8] and others, and it can now be regarded as an established fact that the occurrence of eosinophilia is one of the most constant and diagnostically important symptoms of the disease. That it does not occur invariably, however, is shown by the reports of Howard, Da Costa, Drake, and Cutler.[9] A very interesting case of trichinosis is reported by McCrae,[10] in which the disease was complicated by typhoid fever; the eosinophilia was here nevertheless well marked.

In *filariasis* also eosinophilia may occur. As the result of his study of four cases of the disease in the Philippines Calvert[11] concludes that in the early stages hyperleucocytosis with an increase of the eosinophiles may be looked for, but that the number of the leucocytes in general, as also of the eosinophiles, returns to normal as the disease progresses. In one of his cases the percentage increased to 22, but varied within twenty-four hours between this point and 8. In a case of long standing which I had occasion to examine I found but 2 per cent. of eosinophiles, with 36 per cent. of lymphocytes. Calvert, on the other hand, noted no increase of the lymphocytes. A

[1] Bückler, Münch. med. Woch., 1894, Nos. 2 and 3.
[2] Strong and Musgrave, Johns Hopkins Hosp. Bull., 1901.
[3] Amberg, "Amœbic Colitis in Children," Johns Hopkins Hosp. Bull., 1901.
[4] Brown, Jour. Exp. Med., vol. iii. p. 315; and Johns Hopkins Hosp. Bull., 1897.
[5] Thayer, Phila. Med. Jour., vol. i. p. 654.
[6] Cabot, Boston Med. and Surg. Jour., vol. cxxxvii. p. 676.
[7] Centralbl. f. Bakt., vol. xxv. p. 746.
[8] Blumer-Neumann, Am. Jour. Med. Sci., vol. cxix. p. 14.
[9] Cutler, Trans. Assoc. Am. Phys., 1902, p. 356.
[10] McCrae, Am. Jour. Med. Sci., 1902, vol. cxxiv. p. 56.
[11] Calvert, Johns Hopkins Hosp. Bull., 1902, vol. xiii. p. 133.

relation between the number of embryos and the percentage of the different leucocytes does not appear to exist.

Eosinophilia has further been noted in *hydatid disease.* Seligmann and Dudgeon[1] thus report a case of hydatid disease of the liver in which there was a leucocytosis of 17,111 with 57 per cent. of eosinophiles. After operation the leucocytes diminished to 7000 and the eosinophiles to 1 per cent. A similar case has been reported by Bloch[2] with 14.7 per cent. of eosinophiles, and it is noteworthy that the cyst at the time was undergoing suppuration. Four weeks after operation normal values were obtained. In three other cases of hydatid disease reported by Bloch, involving the lung and the spleen, there was no increase of the eosinophiles and in one a marked lymphocytosis.

In *malaria* the eosinophiles are commonly present in increased numbers during the afebrile period, and rarely diminish below the minimum normal values even at the time of a paroxysm. Zappert[3] reports a case of malaria in which on the day following the last attack 20.34 per cent. (1486 absolute) were found.

In *malignant disease* eosinophilia apparently occurs in only a relatively small percentage of cases, and when present is usually of moderate grade—*i. e.*, not exceeding 7–10 per cent. Occasionally, however, the increase is most remarkable. Reinbach thus cites a case of lymphosarcoma (malignant lymphoma) of the neck with metastases in the bone-marrow, in which 60,000 eosinophilic leucocytes were counted on one occasion.

A *gonorrhœal eosinophilia* has been noted by various observers. From an analysis of 45 cases which Owings studied in my laboratory it appears that with an extension of the inflammatory process to the posterior urethra the number of cases increases in which an increased percentage of eosinophiles is found in the blood, and in cases of prostatitis eosinophilia is the rule. During the first week of the disease the blood is apparently always normal. In the second and third weeks it is normal in only 33 per cent. of all cases, and after two months' duration an increased number is still observed in 40 per cent. The percentage of the eosinophiles usually does not exceed 12 per cent. At times, however, larger numbers are found; Bettmann cites a case of gonorrhœal epididymitis with 25 per cent. Occasionally the eosinophilia is associated with a neutrophilic hyperleucocytosis; this is usually of moderate intensity, but may be quite marked when the urethritis is complicated by an epididymitis, an orchitis, or a cystitis.

In association with chronic *tumors of the spleen* and after extirpation of the organ eosinophilia has been repeatedly observed. Müller

[1] Seligmann and Dudgeon, Lancet, June 21, 1902.
[2] Bloch, Deutsch. med. Woch., No. 29, 1903.
[3] Zappert, Zeit. f. klin. Med., vol. xxiii. p. 227.

and Rieder[1] report three cases of tumor referable to congenital syphilis, hepatic cirrhosis, and neoplasm of the cranial cavity, in which 12.3, 7, and 6.5 per cent., respectively, were found. After extirpation of the spleen eosinophilia is not immediately observed, but develops only after many months and is of moderate grade.

As I have pointed out, the eosinophilic leucocytes are relatively diminished and may disappear altogether in the great majority of the acute infectious diseases, with the exception of scarlatina perhaps, while hyperleucocytosis referable to the polynuclear neutrophilic cells exists. In the post-febrile period, however, the upper limit of the normal and even a well-marked eosinophilia are often observed. Türk[2] thus found an *epicritic eosinophilia* of 5.67 per cent. (430 absolute) in a case of pneumonia, and after an attack of acute articular rheumatism 9.37 per cent. (970 absolute). I have recently seen an eosinophilia of 10.5 per cent. after pneumonia.

An *eosinophilia referable to drugs* has been described, but has attracted little attention. Two cases are reported by v. Noorden, who observed an increase of the eosinophiles to 9 per cent. Both were cases of chlorosis, and in both the eosinophilia followed the internal administration of camphor. Similar observations have been made in animals after poisoning with carbon dioxide.

Following the injection of tuberculin an increase of the eosinophiles has been observed in those cases in which a febrile reaction had occurred. In one case reported by Grawitz the eosinophilia reached its highest point, viz., 41,000, three weeks after the injections had been stopped.

Polynuclear Eosinophilic Hypoleucocytosis (Hypo-eosinophilia).—A diminution in the number of the eosinophiles is notably observed in the acute infectious diseases which are associated with a neutrophilic hyperleucocytosis. The only exception to this rule apparently is scarlatina; but here also their number is diminished at the onset of the fever, and, as I have stated, in fatal cases they rapidly disappear. Aside from the infections which lead to an increase of the polynuclear neutrophiles, hypo-eosinophilia also occurs in those forms which, like measles and typhoid fever, are associated with a decrease of the leucocytes. We may accordingly formulate the general rule that a diminution in the number of the eosinophiles will be observed at some period in the course of the various acute infectious diseases, no matter whether they are associated with a general polynuclear hyperleucocytosis or not. The extent to which this may go is variable; in the milder infections the values may be but little, if any, below the minimum normal, but in the severer and more protracted cases not a single eosinophile may be met with in a

[1] Müller and Rieder, Deutsch. Archiv, vol. xlviii. p. 105.
[2] Türk, Klinische Blutuntersuchungen, Wien, 1898.

differential count of a thousand. Whether or not cases occur in which they are wholly absent I am not prepared to say.

Aside from the acute infectious diseases it is uncommon to meet with a material diminution of the eosinophiles. It has been observed after severe muscular exercise and after castration, and it is commonly noted in lymphatic leukæmia with high lymphocyte counts. DaCosta[1] states that he has found a decrease or even an absence of eosinophiles in the majority of cases of chlorosis and pernicious anæmia. This decrease in pernicious anæmia has also been observed by others,[2] and is apparently the rule during the active stage of the diseases; in the interval, however, normal and even supernormal values may be obtained.

The most extensive diminution of eosinophiles which I have personally observed occurred very curiously in a case of myelogenous leukæmia; but two eosinophiles were seen during a differentiation of many thousand cells.[3]

Lymphocytosis.—According to Ehrlich's conception of lymphocytosis as a *passive* hyperleucocytosis, an increased number of lymphocytes will be found in the blood whenever an increased circulation of lymph occurs in more or less extensive lymphatic districts, the lymphocytes being mechanically washed into the blood current. But, as I have pointed out, there is evidence to show that the lymphocytes also may follow the laws of chemotaxis, and that an active lymphocytosis may possibly occur which is quite analogous to the hyperleucocytoses referable to the polynuclear granular elements.[4]

Under physiological conditions an increased number of lymphocytes is notably observed in early childhood. Following the temporary increase of the polynuclear neutrophiles which occurs during the first twenty-four hours, the lymphocytes rapidly increase in number, so that by the twelfth day they represent 45 per cent. of all leucocytes (Carstanjen). Gundobin gives 59 per cent. as an average value for sucklings as compared with 34.6 per cent. of polynuclear neutrophiles. In adult life a physiological increase of the lymphocytes is notably seen in connection with the increase of the polynuclear neutrophiles which occurs during the process of digestion.

Under pathological conditions lymphocytosis is more common in children than in adults, and it is noteworthy that in anæmic and poorly developed children the normal ratio of lymphocytes to the polynuclear neutrophiles is reached only late. As a general rule the increase of the lymphocytes is not excessive and does not raise the

[1] Clinical Hæmatology, Blakiston, Phila., 1901.
[2] Strauss u. Rohnstein, loc. cit., p. 31.
[3] Simon, Am. Jour. Med. Sci., June, 1903.
[4] Jolly, "Sur les mouvements amœboides des globules blancs," etc., Compt. rend. de la soc. d. biol., 1898, vol. x. série v.; and Wolff, "Giebt es eine aktive Lymphocytose," Deutsch. Aerzte-Zeit., 1901, No. 18.

total leucocyte count much above 30,000 to 40,000. Lymphocytosis of this order is notably seen in rickets, in whooping-cough, measles, congenital syphilis, in various subacute intestinal disorders of childhood, at times in bronchopneumonia, etc.

In *whooping-cough* during the convulsive stage the total number of the leucocytes may be increased to four times the normal number; the average in De Amicis and Pacchioni's[1] series was 17,943. According to these observers, the hyperleucocytosis is demonstrable on the first day of the disease; it reaches its highest point in the convulsive stage and persists some time after cessation of the typical symptoms. Wanstall[2] in his series of 16 cases, on the other hand, finds no evidence of a marked general hyperleucocytosis, and reports that in some the leucocytes were actually decreased. He could demonstrate a well-marked lymphocytosis during the catarrhal stage, however, in almost every case, which varied between 40 and 60 per cent. Wanstall concludes that an increased percentage of lymphocytes, at least equalling if not exceeding that of the polynuclear neutrophiles, is a valuable aid in the diagnosis of whooping-cough before the characteristic symptoms of the disease have appeared. Exceptions, however, occur, in which the lymphocytosis does not reach the usual high figures.

In *rickets* a well-marked lymphocytosis is the rule, which is both relative and absolute; the same holds good for *congenital syphilis* and for the secondary stage of the acquired disease.

In *bronchopneumonia* there is at times a well-marked lymphocytosis instead of a polynuclear hyperleucocytosis. Cabot cites an instance with a total leucocyte count of 94,600 and 66 per cent. of lymphocytes.

In *measles* there is at first an increase of the polynuclear neutrophilic elements; later the lymphocytes increase in inverse proportion to the neutrophiles, the total number being largely dependent upon the degree of glandular involvement.

In *typhoid fever* a relative lymphocytosis begins about the end of the first week and reaches its highest point in the stage of defervescence (see page 96). Ewing states that he has found a uniform relation in this disease between the lymphocytosis in the blood and the grade of lymphatic hyperplasia found at autopsy. He records an instance in which the examination of the blood led to a strong suspicion of lymphatic leukæmia, and in which at autopsy the mesenteric glands were of unusually large size, and the edges of the partly necrotic intestinal ulcers rose 1.5 cm. above the mucosa.

In uncomplicated cases of *pseudoleukæmia* an absolute increase of the leucocytes does not occur; but there is usually a relative increase of the lymphocytes of such extent that the normal ratio to the

[1] De Amicis and Pacchioni, Clin. Med. Ital., 1899, No. 1.
[2] Wanstall, Am. Med., 1902.

polynuclears 1 : 3 rises to 2–3 : 1. This relative lymphocytosis Ehrlich and Pinkus regard as characteristic of true pseudoleukæmia in the differential diagnosis from sarcomatosis and other lymphomatous growths.[1] Grawitz,[2] on the other hand, maintains that from the leucocyte count no diagnostic conclusions can be drawn, and cites cases in which the ratio was either normal or in which the lymphocytes were actually diminished.

The highest grade of lymphocytosis is met with in the so-called *lymphatic form of leukæmia*. As in the myelogenous variety, the total number of the leucocytes is here also very much increased, though not to the same extent. The highest count in Cabot's series was 220,000 and the lowest 40,000, so that we may regard 130,000 as an average. The lymphocytes usually number more than 90 per cent. In the chronic cases the small lymphocyte prevails, while in the acute cases the large lymphocyte controls the blood picture. When septic complications develop, the total number of the leucocytes, as in the myelogenous form of leukæmia, likewise undergoes a considerable diminution, but the lymphocytes still remain relatively increased. In one case of Cabot's, in which as the result of septicæmia the total number of leucocytes fell to 471 per cbmm., the percentage of lymphocytes still was 94.7.

An *experimental lymphocytosis* has been observed following the injection of tuberculin and of extract of carcinomatous growths (Grawitz). Waldstein claims to have produced a marked increase of the lymphocytes by hypodermic injections of pilocarpin, but, according to Ewing, this increase is only relative and brought about by a diminution of the polynuclear cells. Wilkinson speaks of a lymphocytosis following injections of quinine hydrochlorate, and Perry has noted the same after the administration of thyroid extract.[3]

Lymphopenia.—Lymphopenia is notably observed in the acute infections which are associated with an increase of the polynuclear neutrophiles, and is almost always relative. The condition *per se* has received but little attention, and is as yet unimportant from the clinical standpoint.

Clinical Variations in the Number of the Large Mononuclear Leucocytes.—Variations in the number of the large mononuclear leucocytes are as a rule not sufficiently marked to cause either a distinct increase or decrease of the total number of the leucocytes. One notable exception to this rule, however, exists in the cases of the acute type of lymphatic leukæmia, in which the predominant cell is the large lymphocyte, viz., the juvenile form of the common large mononuclear

[1] Pinkus, Die Leukæmie, Nothnagel's Encykl.
[2] Grawitz, Klinische pathol. d. Blutes, 2d ed.
[3] Cited by Da Costa.

leucocyte, in the sense of Pappenheim. At the same time it must be noted that some cases of chronic lymphatic leukæmia also occur in which the large mononuclear leucocyte and Ehrlich's transition-form represent a large percentage of the leucocytes. These relations, however, are not constant.

In the so-called pseudoleukæmia infantum of v. Jaksch a marked increase of the mononuclear elements is observed in a certain percentage of cases, but in the larger number the general increase of the leucocytes is referable to an increase of the polynuclear cells.

A relative as well as an absolute increase of moderate grade is observed in many of the diseases in which the lymphocytes are increased, as in rickets, syphilis, measles, scarlatina, smallpox, etc. It is often marked in chronic malaria, and is sometimes seen after removal of the spleen. I have observed a marked increase in a case of Addison's disease a few days before death, and found notable numbers in debilitated individuals, in association with sloughing epithelioma, etc.

Clinical Variations in the Number of the Mast-cells.—A small number of mast-cells is found in the blood under normal conditions. The presence of more than 1.5 per cent. is probably always pathological. A remarkable increase is noted in the myelogenous type of leukæmia, and is one of the most constant features of the disease; more constant, in fact, than the increase of the eosinophiles. In the one case which I have reported in which the latter were practically absent the absolute increase of the mast-cells was well marked at the height of the disease. The percentage is not necessarily above normal, but not infrequently values of from 5 to 10 per cent. are found. It is noteworthy that this increase of the mast-cells may be demonstrable at a time when the disease is apparently quiescent; in one instance of this kind the total number of the leucocytes had been 350,000; three months later I counted but 2080, of which 10.9 per cent. were mast-cells.

A more moderate increase is noted in many other diseases. Generally speaking, my experience has been that they are more numerous in conditions in which the eosinophiles are also increased, and are generally diminished when the eosinophiles are below normal. This rule, however, is not absolute. I have found values above the normal in various skin diseases, in gonorrhœa, in certain cases of malignant disease, associated with eosinophilia. In one case of renal carcinoma a few weeks after the removal of the growth I counted more than 2 per cent. of mast-cells, with but 1.9 per cent. of eosinophiles. Immediately before operation the count had been only 0.6 per cent. In a case of carcinoma of the cervix I found 0.9 per cent. of mast-cells, with 10 per cent. of eosinophiles; in an advanced

case of phthisis I found 1.2 per cent. Canon[1] reports an increase of mast-cells in chlorosis; Sherrington,[2] in cases of Asiatic cholera, dying in the reactive stage; Taylor,[3] in 2 cases of septic bone disease; and Da Costa states that an increase has also been observed in some cases of splenic anæmia.

I have found the number diminished or entire absence of mast-cells in some cases of malignant endocarditis, appendicitis, empyema, influenza, tonsillitis, intestinal obstruction, lumbar abscess, periproctitic abscess, pernicious anæmia, hæmatoma of the abdominal walls, diabetes, carcinoma of the cervix (septic), "black" jaundice, pneumonia (unresolved).

Myelocytosis.—At birth and in young children it is usual to meet with a small percentage of *neutrophilic myelocytes* in the circulating blood under perfectly normal conditions. In adult life, however, their presence is always a pathological event. In small numbers they may then be met with under the most diverse conditions. Türk has shown that they are quite common in the acute infectious diseases of childhood, and in diphtheria Engel[4] ascertained that they are especially numerous in the severe cases (3.6–16.4 per cent.). In mild infections they are not usually seen, and when present they are found in only very small numbers. In pneumonia they are absent or very few in number at the beginning of the disease, while at the time of the crisis or immediately thereafter they become more numerous and in some cases represent 12 per cent. of all neutrophilic cells; such high percentages, however, are rather uncommon and are more apt to be encountered in children than in adults. In acute septic conditions a small number of myelocytes may also be observed; larger numbers are found in the more chronic cases, which are associated with marked anæmia. In a case of lumbar abscess which had been discharging for six months I found 7.8 per cent.

In anæmic conditions of whatever origin it is common to meet with a moderate number of neutrophilic myelocytes. In pernicious anæmia they are quite constant in the active stage of the disease; as a rule the values do not exceed 0.5–1 per cent., but at times they may reach 7 per cent. In the secondary anæmia associated with syphilis and malignant disease, as also in the pseudoleukæmia of v. Jaksch, similar figures are found. In a young child in which a notable anæmia had developed as the result of amœbic dysentery Amberg counted 9 per cent. In the æstivo-autumnal type of malaria they are quite common.

I have found 2.2 per cent. of neutrophilic myelocytes in a case of

[1] Canon, Deutsch. med. Woch., 1892, vol. xviii. p. 206.
[2] Sherrington, Proc. Royal Soc. London, 1894, vol. lv. p. 189.
[3] Taylor, Contribution from the William Pepper Laboratory, Phila., 1900, p. 148.
[4] Engel, Deutsch. med. Woch., 1897, vol. xxiii. No. 8.

"black" jaundice. Neusser has noted their presence in asphyxia and acute mania; Ewing states that they have been found in considerable numbers in rickets, osteomyelitis, and osteomalacia. Da Costa speaks of their occurrence in poisoning by carbon monoxide, in hepatic cirrhosis, acute gout, malignant endocarditis, and exophthalmic goitre.

The neutrophilic myelocytes which are met with under these various conditions are almost without exception of the small trachychromatic variety. The amblychromatic variety is practically only encountered in the myelogenous type of leukæmia, which is really the disease in which large numbers of myelocytes of all kinds find their way into the blood. Upon their presence in numbers exceeding those found in all other diseases the diagnosis is largely dependent. The blood state is that of a true *myelœmia*. The number of neutrophilic myelocytes in myelogenous leukæmia is often most remarkable, and a count of from 50,000 to 100,000 per cbmm. is by no means exceptional. The average percentage of 18 cases reported by Cabot was 37.7, corresponding to a total number of 162,000 leucocytes. Coincidently with the neutrophilic myelocytes eosinophilic myelocytes also appear in the blood and constitute the majority of the eosinophilic cells seen in this type of the disease; their percentage, however, is rarely large. The total number of the polynuclear eosinophiles is at the same time increased, although the relative percentages may be normal or even slightly below normal. The polynuclear neutrophilic cells and the lymphocytes, while absolutely increased, are relatively much diminished. Of the latter, only 7.6 per cent. are found on an average, and of the former 49.2 per cent., as compared with 20–30 and 60–70 per cent., respectively, under normal conditions. The mast-cells, as I have pointed out, are invariably present in increased numbers in the myelogenous type of the disease.

While the majority of the neutrophilic and eosinophilic cells present a normal habitus, it is common in myelogenous leukæmia to meet with dwarfed forms of doubtful origin. Occasionally leucocytes are observed which are undergoing mitosis. Of special interest is the fact that in certain chronic cases of the disease the neutrophilic cells apparently lose the power of forming neutrophilic material. Non-granular polynuclear cells and myelocytes then appear in the blood and may give rise to much confusion in a differential count. In one case of this kind reported by Ehrlich the great majority of the mononuclear elements, which constituted 70 per cent. of the total number, were entirely free from neutrophilic granules.

The total number of the leucocytes in myelogenous leukæmia in the active stage of the disease is much increased. In Cabot's series of 30 cases the average was 438,000. If at the same time, as not infrequently occurs, there is a coincident anæmia with marked

diminution of the red cells the ratio between the whites and reds may fall to 1 : 2 or even 1 : 1 ; there are cases on record, indeed, in which the leucocytes outnumbered the red cells. Formerly much stress was laid upon this ratio in the diagnosis of the disease ; leukæmia was regarded as a hyperleucocytosis in which the ratio exceeded a definite proportion that was generally placed at 1 : 50. As a matter of fact, there is probably no other disease in which so great an increase of the leucocytes is observed, and even at the present day the diagnosis is usually justifiable when an increase of such proportions is noted. But, as I have pointed out, myelogenous leukæmia is essentially a myelæmia, and not a hyperleucocytosis. There are cases, moreover, exceptional to be sure, in which the increase of the leucocyte is not so extreme, and I have myself observed one case in which the total number was only 2080 and the ratio of the whites to the reds 1 : 1015. The diagnosis of the disease should hence be based primarily upon qualitative changes in the morpholgy of the blood and only secondarily upon an increase of the leucocytes as a whole.

When septic complications supervene in the course of the disease, the blood condition may undergo marked changes. Thus, in proportion to the degree of infection the myelæmic picture gradually disappears and is replaced by that seen in simple septic conditions. The polynuclear neutrophiles may then increase to 90 per cent., and even more, while the eosinophiles diminish and may almost disappear.

In the purely lymphatic form of leukæmia neutrophilic myelocytes are scanty ; there are cases of mixed leukæmia, however, in which at some stage of the disease the blood picture is essentially of the lymphatic type, while at another period there is a marked myelocytosis.[1]

Eosinophilic myelocytes, aside from their occurrence in myelogenous leukæmia, are comparatively rare. They have been found in the pseudoleukæmia of infants ; Mendel[2] speaks of their occurrence in a case of myxœdema ; Türk[3] reports that they are occasionally seen in some of the infectious diseases, and Bignami claims to have seen them in pernicious malaria. In one case of post-hemorrhagic anæmia referable to a ruptured tubal pregnancy I found 1 per cent. of eosinophilic myelocytes, and in a case of myelogenous leukæmia in which the eosinophiles were absolutely much diminished, the only eosinophile that I could find in many slides was a myelocyte.

[1] For a detailed consideration of the blood-changes in leukæmia see especially: Pinkus, "Die Leukaemie," Nothnagel's Encycl. Ewing, Clinical Pathology of the Blood, Lea Bros. Cabot, Clinical Exam. of the Blood, Wm. Wood & Co. Pappenheim, Zeit. f. klin. Med. Hæmatologisch Streitfragen, 1903.

[2] Mendel, Berl. klin. Woch., 1896, No. 45.

[3] Türk, Klin. Untersuch. d. Blutes, etc., Wien u. Leipzig, 1898.

The Plaques.

In addition to the leucocytes and red corpuscles large numbers of small roundish elements are encountered in the blood which measure about 3 μ in diameter and are free from coloring-matter (Plate II., Fig. 1). They are frequently seen collected into groups resembling bunches of grapes. These are the blood-plates or plaques of Bizzozero. According to Hayem, they represent red corpuscles in an early stage of development, and are themselves derived from leucocytes within the lymph-channels. He terms them *hæmatoblasts*. This view regarding the origin and fate of the plaques is scarcely shared by any modern hæmatologist. Lilienfeld, Hauser, Howell, and others regard the plaques as disintegration-products of leucocytes, and notably the nuclear portion, while still others look upon them as precipitated globulins derived in part from the morphological elements of the blood and in part originating directly in the plasma. More generally accepted is the view expressed by Engel, Bremer, Maximow, Pappenheim, and others, according to which the plaques are derived from the red cells by extrusion. They are originally contained in the interior of the cells as so-called nucleoids, and represent the remains of the original nucleus, which has lost its individuality as the result of chromatolysis. As a matter of fact, it is possible by suitable staining to demonstrate the plaques not only within the red cells, but also their extrusion from the cells, so that the erythroglobular origin of some of these formations at least can scarcely be doubted. Jost, moreover, has shown that in the blood of sheep and calf embryos they appear at a time when leucocytes are not as yet demonstrable. But, on the other hand, there is a possibility that what we generally designate as plaques really does not represent a unity, and that some of the elements which resemble the true blood-platelets may be of different origin. To a certain extent such ill-defined little bodies are without doubt derived from leucocytes by a process of plasmorhexis—*i. e.*, by the liberation of small bits of protoplasm. This may be observed under the microscope directly.

Deetjen has recently shown that the true plaques are capable of executing amœboid movements when the blood is placed on a slide which has been covered with a thin film of agar containing a certain amount of sodium chloride, sodium metaphosphate, and dipotassium phosphate. He also believes to have demonstrated a nucleus in the individual plaque, and concludes that the bodies in question do not represent artefacts or products of degeneration, but are in reality true cellular elements.

The agar medium which Deetjen employed is prepared as follows: 5 grammes of agar are dissolved in 500 c.c. of distilled water by boiling. The hot solution is passed through a filter, when every 100 c.c. of the filtrate are treated with 0.6 gramme of sodium

chloride, 6 to 8 c.c. of a 10 per cent. solution of sodium metaphosphate, and 5 c.c. of a 10 per cent. solution of dipotassium phosphate. To demonstrate the nucleus in smears, the specimens are fixed from one to two minutes in 96 per cent. alcohol; they are allowed to dry, and are further fixed from three to five minutes in an 0.5 per cent. solution of formalin. They are then washed in water and stained with hæmatoxylin according to Ehrlich's method. I have personally found that in ordinary smears which have been stained with the eosinate of methylene-blue without any special previous fixation the nucleus-like body can also be seen. Quite significant is the fact that with any one modification of the Romanowsky method a distinct chromatin stain can be produced in the plaques.

According to Osler, the number of plaques varies normally between 200,000 and 500,000 per cbmm. Brodie and Russell, on the other hand, claim that this number is too small, and that with their improved method of counting (page 147) an average of 635,300 is obtained. The normal ratio between the plaques and the red corpuscles would thus be 1 : 7.8, taking 5,000,000 as the average normal for the red cells.

Under pathological conditions the plaques may be increased or diminished. Hayem's statement that they occur in greatly diminished numbers in pernicious anæmia has on the whole been confirmed. According to Lazarus, van Embden found 64,000 and 32,000 in two cases, which, accepting the normal figures of Russell and Brodie, would mean a diminution to less than one-twentieth of the normal. At times they may indeed be absent, but in some cases increased numbers are also found (Strauss and Rohnstein). In leukæmia the plaques are often greatly increased. A large increase is at times observed in post-hemorrhagic anæmia and in chlorosis, but the results are not constant. In the secondary anæmias referable to carcinoma, sepsis, tuberculosis, etc., the findings are variable; sometimes an increase is observed, at others a decrease, and then again normal values; the results, moreover, are inconstant in one and the same case. In the acute infectious diseases their number is the smaller the more severe the course of the disease.

To study the plaques in the wet preparation it is necessary to prevent their agglutination by the immediate addition of a drop of Hayem's fluid (page 141), or the reagent suggested by Afanassiew, which has the following composition:

Peptonum siccum	0.60
Methyl-violet	0.01
Physiological salt solution (0.9–0.95 per cent.)	100.00

The reagent must be kept sterile. Either method will answer most purposes, and it is well to puncture the finger through a drop

of the fluid. If it is desired to study the movements of the plaques, Deetjen's method must be employed. In the dry preparation they are most conveniently demonstrated with the eosinate of methylene-blue or one of the modifications of the Romanowsky method.

(For the enumeration of the plaques see page 147.)

LITERATURE.—Bizzozero, Virchow's Archiv, vol. xc. Hayem, Le sang, Paris, 1889. Howell, Jour. of Morph., 1891, vol. iv. p. 57. Maximow, Arch. f. Anat., 1899, vol. i. p. 33. Jost, Arch. f. mik. Anat., 1903, vol. lxi. p. 667. Determann, Deutsch. Arch. f. klin. Med., vol. lxi. p. 365. Deetjen, Virchow's Archiv, 1901, vol. clxiv. p. 239. Brodie and Russell, Jour. Physiol., 1897, Nos. 4 and 5.

The Dust Particles or Hæmokonia of Müller.

These may be seen in any fresh specimen of blood mounted in the usual manner. They are small, generally round, sometimes dumb-bell-shaped, colorless, highly refractive granules, which manifest very active molecular movements. They occur in the plasma of the blood and are apparently not connected with the process of coagulation. Müller found them abnormally numerous in a case of Addison's disease, while they were diminished during starvation and in various cachectic conditions. Stokes and Wegefarth regard these granules as identical with the neutrophilic and eosinophilic granules of the leucocytes. They suppose that the bactericidal power of the leucocytes and of the serum of man and many animals is due to their presence. As a matter of fact, the origin of the hæmokonia from the granular leucocytes can not infrequently be directly observed.

I have quite constantly found the hæmokonia increased at the height of digestion, and have then repeatedly observed their extrusion from both neutrophilic and eosinophilic cells.

LITERATURE.—H. F. Müller, "Ueber einen bisher nicht beachteten Formbestandtheil d. Blutes," Centralbl. f. allg. Path. u. path. Anat., 1896, p. 929. W. R. Stokes and A. Wegefarth, "The Presence in the Blood of Free Granules, etc., and their Possible Relation to Immunity," Johns Hopkins Hosp. Bull., 1897, p. 246. E. B. Sangree, "Leucocytic Granules," etc., Phila, Med. Jour., 1898, p. 472.

General Technique.

Slides and Cover-glasses.—To obtain satisfactory results, it is essential to have glassware of the best quality. The cover-glasses should not measure more than 0.08–0.10 mm. in thickness and must be cleansed with care. The same holds good for the slides, which should have a *level* surface; many of those furnished by dealers are unsatisfactory for work with immersion lenses.

Both covers and slides should be placed in concentrated sulphuric acid or in glacial acetic acid for several hours. They are thoroughly washed in running water and distilled water and then placed in alcohol and finally in ether, where they remain for several hours. During this process care must be had that they are well separated

from each other. Subsequently they are kept in jars with absolute alcohol, and are dried just before use, or they may be dried at once with fine linen or Japanese lens paper and stored in dust-proof receptacles. When once cleansed, the cover-glasses should be handled only with forceps, as the moisture of the hands is in itself sufficient to produce post-mortem changes in the red corpuscles.

To cleanse slides that have been used, the covers must first be removed by immersion for several days in xylol or turpentine. They are then placed in hydrochloric acid to which about a teaspoonful of potassium chlorate has been added for every 30 c.c. The mixture is kept on the boiling water-bath to the point of decolorization. The slides are next rinsed in hot water, heated for a half hour in a thin mush of equal parts of washing soda, sawdust, and talcum, prepared with the aid of water and stirring frequently, then washed off with hot water acidified with hydrochloric acid, and finally with pure hot water, alcohol, and ether.

The Blood Mount.—We distinguish between wet mounts and dry mounts. Wet specimens can only be utilized successfully if the patient is near at hand to the laboratory, as in office-work and in the hospital; where several hours must elapse before the preparation can be examined, it will usually be best to resort to the dry specimen. Wet preparations, however, are very convenient and yield a large amount of information without delay, and a rapid survey will indicate whether or not it will be necessary or advisable to resort to a more detailed examination. The grade of an anæmia, the degree, character, and extent of a hyperleucocytosis, the presence of malarial organisms, can all be told from the wet preparation. With the dry and stained specimen, on the other hand, all these points are brought out more distinctly, and other information is further afforded which cannot be obtained from the wet specimen alone.

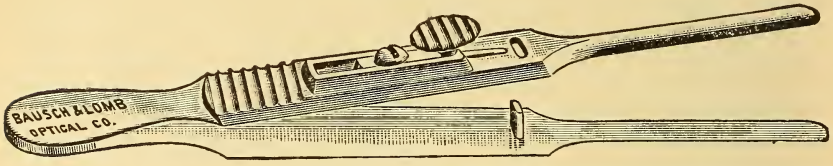
To prepare a blood specimen, the tip of a finger, or in children especially the lobe of the ear, is first cleansed with ether and then punctured with a suitable instrument, such as a fine lancet or a stout needle. The puncture should be sufficiently deep that the blood will flow from the wound without undue pressure.

To prepare a wet specimen, a clean cover-glass is taken up with a pair of forceps, with flat blades and a light spring, touched to the drop without coming in contact with the skin, and immediately transferred to a clean slide. If suitable glassware is used that is perfectly clean, the drop will immediately spread in a circular fashion between cover-glass and slide, and on examining with a low power, which should always precede examination with a high power, it will be noted that in the central portion of the specimen especially the red cells will be well separated from one another and will not have run into rouleaux. This will only occur if the glassware is imperfect, if it is not perfectly clean, or if the drop has been too

large. To gauge the proper size of the drop requires a little practice. Along the margin of the specimen, where a certain amount of evaporation is going on, it is usual to find rouleaux and crenated red corpuscles, even though the rest of the specimen is perfect, and in the course of time post-mortem changes will also become noticeable throughout the preparation. If the specimen is ringed with a little paraffin, however, a satisfactory examination is still possible after a number of hours, and even without being ringed such preparations can be kept for at least one hour.

To prepare dry specimens, which are subsequently to be stained, the blood is spread in a capillary layer between cover-glasses or on slides. In the first case, one cover-glass is locked in a pair of forceps such as those devised by Ehrlich and pictured in the accompanying illustration (Fig. 15). A second cover is taken up with

FIG. 15.



Ehrlich's cover-glass forceps.

a pair of forceps without a lock, but with flat blades and a light spring; this is held to the drop of blood just as it emerges from the puncture, and is then immediately laid upon the first cover. If the glasses are of satisfactory quality and clean, the blood will at once spread in a capillary layer; the top cover is then drawn from the lower cover by grasping the edge firmly with the fingers and making even traction in a plane parallel to the other. Here also a certain amount of experience is necessary in gauging the size of the drop in reference to the size of the covers. In no case should it be so large that the top cover *floats* upon the blood. If the drop is rather small, the two covers should overlap only to such an extent as to furnish a space which is just filled by the blood. If the drop is larger, they should overlap over a larger surface.

The above method is the one originally suggested by Ehrlich, and probably the one most commonly employed for making dry smears. Personally I have almost abandoned the use of cover-glasses, and much prefer slides for routine work. But little practice is required to obtain very satisfactory results, and it is possible to control the quality of the individual smears with a degree of precision which is but rarely attained even by the most experienced workers with cover-glasses. The spreads, moreover, are much larger, so that there will always be a sufficient number of leucocytes available even under normal conditions to permit a count of at least

a thousand cells. At the same time it is possible to spread portions of the drop so thin that the individual cells are well separated the one from the other, while other portions can be made a little thicker. The slides are cleansed in the same thorough manner as in the case of the cover-glasses. A fair-sized drop of blood is then mounted near the end of one slide and spread with an even sweep with the edge of a second slide; this should be done with a light hand, and holding the first slide in the left hand between the thumb and the second and third fingers. The second slide should also be held in this manner, but at an angle of 45 degrees to the first, as shown in the accompanying illustration (Fig. 16). Before commencing the

FIG. 16.

The preparation of blood-smears on slides.

sweeping movement I let the blood spread along the edge of the second slide by capillary attraction; then I move across, gradually raising the second slide to a vertical position. Pressure must be carefully avoided.

After being allowed to dry in the air the specimens are placed between layers of filter-paper and may then be examined at leisure. If several days must elapse before the examination, it is well to place them, wrapped in filter-paper, in closed jars. Should it be desired to preserve the specimens for a long time—*i. e.*, for months or years—it is best to coat the films with a thin layer of paraffin, which later is dissolved by immersion in toluol. In this manner especially valuable and rare specimens may be kept almost indefinitely. Unless this precaution is taken, the staining qualities of all the morphological elements of the blood will undergo changes which render the specimens unfit for color analysis.

Fixation.—Before staining, it is frequently necessary to fix the blood-films, to which end several methods may be employed. The best results are usually obtained by heat. For this purpose a copper plate may be used measuring about 10 cm. in width by 40 cm. in length and 3–5 mm. in thickness; this is heated by a Bunsen burner or a small coal-oil stove. After the plate has a fairly constant temperature, the desired degree is ascertained by a series of drops of water, toluol (boiling-point, 110°–112° C.), or xylol (137°–140° C.), etc., noting the line at which ebullition occurs. If the distance of the plate from the flame and the size of the flame, etc., are constant, the apparatus requires practically no attention and serves its purpose very well. As a rule a brief fixation only is necessary—*i. e.*, exposure to a temperature of from 100° to 126° C., for one-half to two minutes, while in special cases Ehrlich recommends a more prolonged exposure or a higher temperature. Very good results are obtained for most purposes by heating the blood-films to a temperature of 140° C. for thirty to forty-five seconds, as suggested by Rubinstein. This point is conveniently ascertained on the copper plate by noting the line at which the so-called Leidenfrost phenomenon begins to occur, viz., the point at which a drop of water assumes the spherical form and rolls about on the plate.

In the place of the copper plate an ordinary drying-oven provided with a thermostat and thermometer or a so-called Victor Mayer Siedekessel may also be employed. The latter is a small copper kettle covered with a thin plate, which is perforated for the reception of the boiling-tube. If a small quantity of toluol is boiled in this kettle for a few minutes, the copper plate will acquire a temperature of from 107° to 110° C., and retains this sufficiently long for ordinary purposes (Ehrlich).

Absolute alcohol or a mixture of equal parts of absolute alcohol and ether (Nikiforoff) have also been recommended as fixing agents for blood-films, but are not very satisfactory for the study of the neutrophilic granulation. With Ehrlich's triacid stain especially it will frequently be noted that the granules are stained imperfectly or not at all. For the study of nuclear structures, however, both are quite satisfactory. In the case of absolute alcohol alone immersion of the blood-films for a few minutes is sufficient; with alcohol and ether fixation for one-half to two hours is necessary.

Formalin is useful as a fixing agent and may be used in connection with practically all the common blood-stains. A 1 per cent. alcoholic solution is employed. This is prepared by diluting one part of the commercial formalin, which is a 40 per cent. solution of formaldehyde in gas, water and methyl alcohol, with nine times its volume of water, and one part of the resulting solution with nine times its volume of alcohol. Fixation is completed in one minute, and for practical purposes it is merely necessary to cover

the blood-films with a few drops of the solution, which is then drained off and replaced with the staining reagent directly.

With certain staining reagents, such as Jenner's eosinate of methylene-blue, or with Leishman's method, previous fixation is not necessary, as the films are here fixed by the methyl alcohol during the process of staining.

The Anilin Dyes and the Principles of Staining.—The anilin dyes with which we have to deal in the clinical laboratory are all derivatives of hydrocarbons and for the most part of hydrocarbons of the aromatic series. Their staining properties are dependent upon the presence in the individual compounds of two distinct atomic complexes which are spoken of as *chromophoric* and *auxochromic* groups, respectively. The presence of the chromophoric group imparts chromogenic properties to the substance, the dye itself resulting on the further introduction of an auxochromic group. The auxochromic groups are salt-forming radicles and render the dye either basic or acid. Two markedly auxochromic radicles are known, viz., the strongly basic amido group —NH_2 and the feebly acid hydroxyl group —OH. Still other salt-forming radicles may enter into the composition of the dye, but it is noteworthy that these have but feebly developed auxochromic properties. Radicles of this order are notably the carboxyl group —COOH, the sulphoxyl group —SO_2OH, the nitro group —NO_2, and the nitroso group —NO (which two latter may also occur as chromophoric radicles). These groups are essentially of influence upon the reaction of the dye, and as the chromophoric radicle itself may have acid or basic tendencies it is manifest that the ultimate reaction of the individual compound will depend upon the inter-relation of acid and basic radicles. Markedly acid dyes will result if both the chromophoric group and the salt-forming radicles are acid, while strongly basic dyes will be the outcome if both have basic tendencies. Between these two extremes various possibilities exist, the ultimate reaction depending upon the character of the chromophore, the presence of acid or basic salt-forming radicles, the simultaneous presence of both, their number, etc. We may accordingly divide the various dyes into the following classes:

1. Basic amido dyes.
2. Acid nitroso dyes.
3. Acid sulpho- and nitro-dyes, viz., amido- or oxysulphonic acids, amido-oxysulphonic acids, nitrophenols, nitroamins, nitro-amidosulpho acids, nitro-oxysulpho acids, nitroamido-oxysulpho acids.
4. Acid oxy- and oxycarbonic dyes.
5. Amido-oxy-, amidocarbonic, and amido-oxycarbonic dyes.
6. Amidosulphocarbonic-, oxysulphocarbonic-, amido-oxysulphocarbonic-, amidonitrocarbonic-, oxynitrocarbonic-, amido-oxynitrocarbonic-, and amido-oxysulphonitrocarbonic dyes.

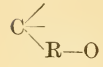
Of chromophoric groups, some twenty are known, and it is customary to classify the anilin dyes on the basis of these underlying radicles. We thus find:

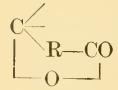
The $—NO_2$ group in the nitro dyes (picric acid, Martius yellow, naphtol-yellow S, aurantia).

The —NO group in the nitroso dyes (Echtgrün naphtol-green).

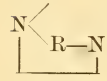
—N=N— in the azo dyes (anilin-yellow, chrysoidin, vesuvin, Sudan G and III, alizarin-yellow FS, Ponceau, Bordeaux, amaranth, coccinin, orange G, tropæolin, Biebrich scarlet, congo, benzopurpurin):

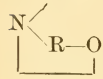
in the rosanilins (malachite-green, brilliant green, methyl-violet, methyl-green, fuchsin, acid fuchsin, iodine-green, anilin-blue, alkali-blue, water-blue, aldehyde-green).


in the rosolic acid dyes (aurins).


in the phthalëins (eosin, spriteosin, erythrosin, phloxin, rose bengale, rhodamin, gallein, cœrulëin).

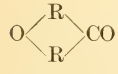
in the anthraquinons (alizarin, purpurin, anthragallol, alizarin-blue).


in the indamins (phenylene-blue, Bindschedler's green, toluylene-blue).


in the indophenols (indophenol-blue).

in the Lauth dyes (Lauth's violet or thionin, methylene-blue, methylene-red, methylene-green).

in the azins (eurhodin, eurhodol, toluylene-red, the safranins, Magdala red, mauvëin).


in euxanthinic acid and possibly in galloflavin (jaune indienne).

in the quinolins and acridins (cyanin, quinolin-red, quinolin-yellow, acridin-red and scarlet).

The majority of the anilin dyes are found in the market in the form of salts of the respective staining acids and bases, and it is noteworthy that the latter as such are for the most part either

colorless or but feebly stained. Triamidotriphenylcarbinol is thus colorless, while its monacid salts are red (fuchsin); phenolphthalëin likewise is colorless, but forms red salts with the alkalies; fluorescëin is pale yellow, but forms the bright-red fluorescent uranin with alkali, etc. The phenols and nitrophenols, however, are commonly used as free acids.

During the process of staining, the salts of the staining acids or bases are decomposed by the animal or vegetable tissue and new compounds result between the free staining acid or base and the various chemical components of the tissue in accordance with the reaction of its component parts. The acid nuclear substance of cells thus shows a special affinity for basic dyes, and the basic protoplasm for acid dyes. Contrasted with this chemical process of staining is the physical process in which the dye is merely stored in the pores of the tissue. Both must be sharply differentiated the one from the other in attempting to draw inferences in reference to chemical affinity on the part of component parts of a tissue or a cell.

While in former years simple dyes were commonly employed in the clinical laboratory and tissues were stained *successively* if more than one dye was used, it has been shown that it is possible to combine acid dyes with basic dyes in such manner that the acid affinities of the one become completely saturated by the basic affinities of the other. *Neutral dyes* thus result in which the staining possibilities of the two original components are not only preserved, but in which additional staining properties have also developed which are the expresssion of their neutral reaction. Stains of this order with well-developed polychrome properties are, of course, exceedingly valuable, as they readily permit an insight into the structure and to a certain extent into the chemical composition of cells which is otherwise only obtained with much difficulty. The credit of having first prepared and used such neutral dyes belongs to Ehrlich, whose triacid stain has been and is still one of the most important differential dyes employed in the clinical laboratory.

The principle underlying the formation of neutral dyes of this character is well shown in the case of the triacid stain. Three single dyes enter into its composition, of which two are acid dyes, viz., acid fuchsin and orange G, while the third, methyl-green, is a basic dye. This latter contains three basic groups which may be saturated by corresponding acid groups. The term "triacid" has thus not reference to the presence of three acid dyes, but to the fact that the three basic radicles of the basic component of the dye have been saturated in the manner indicated. In the present instance these three radicles have not been neutralized by one acid dye, but by two. As a result we have present in the same mixture a fuchsinate of methyl-green and the corresponding neutral compound of the orange G and methyl-green. Solutions of both can be directly mixed, the

one with the other, which is always possible with solutions of neutral dyes if the two solutions to be mixed have one component in common.

While the simple dyes, both basic and acid, are soluble in water, the neutral dyes are practically insoluble, but soluble in an excess of either the acid or the basic component, and more especially the former. If then an aqueous solution of methyl-green is added carefully to an aqueous solution of acid fuchsin, fuchsinate of methyl-green is formed at once, but at first remains in solution owing to an excess of the acid dye. Upon the further addition of methyl-green, however, and standing, a point is reached when the fuchsinate separates out, and if the amounts of the two components have been carefully determined beforehand the filtrate may be nearly colorless. If then an excess of methyl-green is added, a certain amount of the fuchsinate will redissolve; and if the excess be sufficiently great, the entire precipitate will pass into solution.

Aside from an excess of the acid or basic component of the neutral dye its solution can also be brought about in other ways, as with alcohol (notably methyl alcohol), acetone, methylal, etc.

Not all simple dyes are equally well adapted for the preparation of neutral dyes. Of basic dyes, the most useful are those which contain the so-called ammonium group, notably methyl-green, methylene-blue, amethyst-blue, and to a certain extent also pyronin and rhodamin; of acid dyes, the readily soluble salts of the polysulphonic acids, such as orange G, acid fuchsin, and narcëin, and of the salts of the carbonic acids eosin.

Neutral mixtures may then be prepared which contain two or more component dyes. If it is desired to prepare a tricolor mixture, two possibilities suggest themselves, viz., a mixture containing one acid dye and two basic dyes, or one with one basic dye and two acid dyes.

The principle of staining with neutral dyes is the same as in the case of the simple acid or basic dyes. Taking the leucocytes, for example, the nucleins will be found to decompose the neutral body and to unite with the basic component; the eosinophilic granules similarly decompose the dye, but take up the acid component, while in the case of the neutrophilic granules we may imagine that no decomposition is here effected, but that the neutrophilic material unites directly with the neutral molecule.

The number of neutral dyes in use in the clinical laboratory is as yet small; several are modifications of Ehrlich's original triacid. In Pappenheim's two triacid mixtures methylene-blue is used as the base in the one, and methylene-azure as the base in the other.

To the class of the neutral dyes also belongs the eosinate of methylene-blue and the eosinate of methylene-azure which form the basis of the various eosin-methylene-blue solutions originally sug-

gested by Romanowsky, Ziemann, Chenzinsky, Plehn, Ehrlich, Michaelis, etc. The eosinate of methylene-blue solution is commonly spoken of as Jenner's stain; the eosinate of methylene-azure forms the basis of the various modifications of the Romanowsky stain, such as those suggested by Nocht, Ziemann, Leishman, Reuter, etc. All these stains are of the utmost value in the morphological study of the blood more particularly, and although many are merely modifications the one of another, I have described their preparation and uses in detail, for one will frequently serve to bring out a special point which is only well shown in this particular manner. The selection of the individual method will, of course, depend upon circumstances. For routine work I suggest the eosinate of methylene-blue in Jenner's modification, Ehrlich's triacid, Pappenheim's triacid No. 2, Ziemann's method, and Wright's modification of Leishman's method; for the study of malarial organisms aside from the routine stains (excepting Ehrlich's triacid), notably Nocht's stain, Reuter's stain, and Futcher's stain. For the study of the oxyphilic granulation Ehrlich's triglycerin mixture is essential; for the investigation of mast-cell granules Ehrlich's dahlia, and the various violet basic anilin dyes; for the neutrophilic granulation besides the usual routine stains mentioned, also Ehrlich's neutral mixture; for the study of the lymphocytes Pappenheim's pyronin-methyl green, while for the study of nuclear structures the old eosin-hæmatoxylin method still is unsurpassed.

Methods of Staining.

General Methods.—The Eosinate of Methylene-blue (Jenner).[1]—Equal parts of a 1.2–1.25 per cent. *aqueous* solution of eosin and a 1 per cent. *aqueous* solution of methylene-blue are mixed in an open basin and allowed to stand for twenty-four hours. The resulting precipitate—the eosinate of methylene-blue—is washed with water, collected on a filter, dried at a moderate temperature, and finely powdered. The dye can then be stored in bottles and is perfectly stable. For staining purposes an 0.5 per cent. solution in *absolute* methyl alcohol is employed; this can be used at once and keeps indefinitely. I have used this stain as a routine stain for nearly four years and can speak definitely of its value. It has material advantages over the triacid stain as special fixation is not required and as its power of differentiation is more extensive. Its preparation also is simpler, though it is necessary to observe certain precautions. In preparing the dye I first weigh out the requisite amount of eosin and methylene-blue. The eosin is placed in a mortar or evaporating-dish and rubbed into a paste with a small amount of water; more water is then added until all the dye is well dissolved. This solution is poured into a large saucepan and diluted

[1] Jenner, Lancet, 1899, vol. i. p. 370. Simon, Maryland Med. Jour., April, 1900.

to the proper point. The methylene-blue is now similarly brought into solution, though with a little more difficulty as the dye is inclined to be lumpy; it must all be dissolved. This solution is poured directly into the eosin solution and the requisite amount of water further added. The mixture is stirred with a rod and left to stand. If the proper quantities have been used and well dissolved, the filtrate is but little colored, in which case not much washing is necessary; if, however, there is a distinct excess of either dye, and notably the methylene-blue, this must be washed out, which is best done by decantation. The alcoholic solution finally is prepared by rubbing up the dye with the alcohol in a porcelain dish. *Absolute methyl alcohol must be used.*

The blood-films (on slides) are not fixed before staining; this is accomplished by the absolute alcohol during the staining. The specimens are well covered with the stain and after about five minutes washed off with water and dried in the air or by heating moderately over a flame. Care should be had during the staining that the preparations are thoroughly covered with the dye, as otherwise some of the stain is apt to become precipitated as the result of evaporation. After drying, the specimens can be examined directly in a drop of cedar oil. Permanent mounts are prepared by placing a drop of Canada balsam on the specimen and covering it with a cover-glass. With the precautions stated and by strictly adhering to the method as described even the beginner can obtain perfect results. For routine purposes I can recommend the stain without reserve. The differentiation is excellent and most extensive (see Plates III., IV., and VI. especially). The red corpuscles are stained a terra cotta, the nuclei of the leucocytes and nucleated red cells blue, the plaques mauve, the neutrophilic granules a purplish red, the eosinophilic granules bright red, and the mast-cell granules dark violet. Granular degeneration and polychromasia of the red cells is well shown (Plate III.). Malarial organisms, bacteria, and filariæ are stained blue.

Ehrlich's Triacid Stain.[1]—The preparation of a reliable triacid stain, according to Ehrlich, presupposes the use of chemically pure dyes, such as those prepared by the Actiengesellschaft für Anilinfarbstoffe of Berlin. Saturated aqueous solutions of orange G, acid fuchsin, and methyl-green are first prepared and allowed to clear by standing for at least one week. It is essential that these solutions should be perfectly clear, and it is well in measuring off the requisite quantities to remove the supernatant portion with a pipette. The various ingredients are then mixed in a clean bottle, making use of the same measuring-glass, and without washing between the addition of the individual components. These are taken in succession as shown below, and after adding the methyl-

[1] Ehrlich-Lazarus, Die Anæmie, loc. cit.

green the mixture is thoroughly stirred until the remaining portion of alcohol and glycerin has been added.

Orange G solution	13.0–14.0 c.c.
Acid fuchsin solution	6.0– 7.0 c.c.
Distilled water	15.0 c.c.
Absolute alcohol	15.0 c.c.
Methyl-green solution	12.5 c.c.
Absolute alcohol	10.0 c.c.
Glycerin	10.0 c.c.

The solution is ready for use at once and does not deteriorate with age.

In order to obtain the best results with the stain, it is practically necessary to fix the blood-films by heat; fixation by Nikiforoff's method does not furnish constant results, and only too often leaves the neutrophilic granules unstained or imperfectly stained. Fixation at a high temperature (140° C.), as suggested by Rubinstein furnishes better results than the lower temperatures originally advised by Ehrlich, as the difference in color between the neutrophilic granules and the eosinophilic granules is thus brought out more prominently. The blood specimens are stained about five minutes, then washed in water, dried (by blotting, if desired), and examined as usual.

In properly stained specimens the eosinophilic granules present a copper or a yellowish-red color, while the neutrophilic granules are violet. The mast-cell granules remain colorless and appear as round vacuoles in the faintly bluish-greenish protoplasm. The nuclei of the leucocytes present a greenish color and are not well stained. The red cells in properly heated specimens are orange; if the temperature was too high, they are yellow, and in such a case it will be found that their structure has suffered as a consequence. If the temperature has been too low, the red cells take on the fuchsin. The nuclei of the normoblasts are intensely stained; the older nuclei appear black; megaloblastic nuclei, on the other hand, are rather feebly stained, and in some specimens, indeed, the inexperienced will at first sight not discern any nucleus. Granular degeneration is not shown and polychromatophilia cannot be demonstrated so well with the triacid as with the eosinate or in hæmatoxylin-eosin preparations. Malarial organisms are imperfectly shown. The differentiation with the triacid is thus markedly less than in the case of the eosinate. This is owing to the peculiar character of the methyl-green, which is a specific nuclear dye. To counteract some of these deficiencies, Ehrlich has suggested to stain the preparations for a few seconds with an aqueous solution of methylene-blue first, and to stain with the triacid afterward. This improves the pictures somewhat, but it is not wholly satisfactory. As a routine stain in the clinical laboratory Ehrlich's triacid is at present less commonly employed;

in the special study of the neutrophilic granulation, however, it still retains its usefulness.

Pappenheim's Triacid Stain (No. I.).[1]—This, as well as Pappenheim's triacid No. II., has been devised to utilize the advantages of a tricolor mixture while obviating the disadvantages attaching to methyl-green as a base. It is prepared as follows: 1 part of methylene-blue is dissolved in a small volume of water and treated with a concentrated aqueous solution of acid fuchsin, drop by drop, until a precipitate forms; the addition of the fuchsin solution is then continued while stirring until the precipitate is again dissolved, an excess being carefully avoided. The resultant solution is termed A. A second solution B is prepared in a similar manner, but starting with 4 parts of methylene-blue and using a concentrated aqueous solution of orange G as the acid dye. The two solutions are then mixed and the specimens stained as with Ehrlich's triacid after being fixed by heat. They are washed in water, dried, and examined as usual.

The red cells are orange, all nuclei blue, the neutrophilic granules a purplish violet, the eosinophilic granules red, and the mast-cell granules a bluish violet. Granular degeneration and polychromatophilia are well shown and the malarial organism also is stained.

Pappenheim's Triacid Stain (No. II.).[2]—This contains the active principle of Unna's polychrome methylene-blue as base, which Michaelis claims to have identified as methylene-azure, and which he terms azure blue. The dye is best purchased, already prepared, from Grübler, where it is sold under the name of Pappenheim's "panoptic triacid solution." It is used in the same manner as Ehrlich's triacid, after previous fixation of the specimens by heat, but must always be freshly prepared in aqueous solution. It is recommended to give the nuclei a preliminary stain with a concentrated aqueous solution of toluidin-blue, after which the dye is washed off and replaced directly by the triacid.

The advantages of this stain are referable to the peculiar selective action of the azure blue for nuclear structures, and the possibility of demonstrating the presence of such formations in this manner, where with other methods they cannot be shown.

The nuclei of the lymphocytes and large mononuclear leucocytes and of the malarial organisms are colored a bright red, those of the polynuclear leucocytes a bluish violet, and the erythroblastic nuclei almost black. The bodies of the lymphocytes and the malarial organisms are sky blue, the erythrocytes fuchsin-red or orange (according to the degree of heat employed during fixation), the neutrophilic granules violet red, the eosinophilic granules a bright scarlet, and the mast-cell granules carmine. In this manner the

[1] Pappenheim, Deutsch. med. Woch., 1901, vol. xxvii. p. 799.
[2] Pappenheim, Ibid.

presence of chromatin can also be demonstrated in the plaques, which for the most part present a red nucleus surrounded by a pale-blue protoplasm.

The Romanowsky Method.[1]—The history of the Romanowsky method is intimately associated with the study of the minute structure of the malarial organism, in which the presence of a nucleus was first demonstrated by its aid. The dye is essentially an eosin-methylene-blue mixture, the specific staining action of which is, however, not due to the methylene-blue *per se*, but to a decomposition-product of the methylene-blue, viz., the methylene-azure. As I have stated, this also forms the base of Pappenheim's triacid No. II. It apparently combines with eosin to form a neutral dye analogous to the eosinate of methylene-blue, and can similarly be employed as a routine blood stain in the clinical laboratory. As a rule we do not employ solutions of the pure dye, but solutions of methylene-blue containing a variable amount of the methylene-azure, to which the requisite amount of eosin is at each time added. In a mixture of this kind the neutrophilic material is then probably stained *in statu nascendi.*

Reuter has attempted to prepare a stable solution of the eosinate of methylene-azure (with eosinate of methylene-blue) in methyl alcohol, with the addition of oil of anilin, analogous to Jenner's eosinate of methylene-blue, but unfortunately the solution keeps for only a few weeks. After that time so much of the specific dye separates out that not sufficient remains in solution to produce the red color in the nuclei of malarial organisms, even though the nuclei of the white cells are still stained red.

Giemsa was the first to devise methods for the economical preparation of methylene-azure in pure form, which can be obtained from Grübler; in his method an aqueous solution of the azure is directly combined with an aqueous solution of eosin, the two being freshly mixed at each time. Here also the neutrophilic material is then stained by the eosinate of methylene-azure *in statu nascendi.*

The following modifications of the original Romanowsky method are based in principle upon the above considerations:

Nocht's Method.[2]—Two standard solutions are employed, viz., a 1 per cent. aqueous solution of eosin which before use is diluted with 20 to 50 times its volume of water, and a solution of methylene-azure in methylene-blue which is prepared as follows: 100 c.c. of a 1 per cent. aqueous solution of methylene-blue are treated with the precipitated oxide of silver obtained from 1 gramme of silver nitrate by dissolving this amount in water, precipitating with caustic alkali solution, and washing. After standing for four or five days at

[1] Romanowsky, St. Petersburg. med. Woch., 1891.

[2] Nocht, Encyk. d. mik. Tech., vol. ii., Urban u. Schwarzenberg, Berlin-Wien, 1903, p. 785.

ordinary temperature the methylene-blue solution has a distinct reddish tone and contains a considerable amount of methylene-azure. Such a solution is almost neutral.

The staining reagents must always be prepared anew by adding azure blue solution to the eosin drop by drop while stirring, until the color of the resultant mixture is the same as that of the azure solution. If the original methylene-blue solution from which the azure blue was prepared was of the strength of 1 per cent., not quite double the amount of the azure solution as compared with the eosin solution will be necessary. As a rule 4 drops of the eosin, 20 c.c. of water, and 6 to 8 drops of the azure solution will be found about the proper proportions; an excess of the azure blue does not matter.

Cover-glass specimens are best used, which should be fixed by heat or by immersion in alcohol and ether. They are stained in concave watch-crystals, specimen side down, so that the precipitate which is invariably formed during the process of staining does not adhere too firmly to the smeared surface. After from seven to ten minutes the preparations are thoroughly washed with water and examined in a drop of water before making mounts in balsam, in order to ascertain whether the staining has been successful, especially in so far as the malarial organisms are concerned. If the nuclei of the large mononuclear leucocytes are colored dark red, it may generally be inferred that the parasites also will be properly stained. In that event the cover-glass is carefully removed from the slide, dried in the air, and mounted in balsam. The latter must be free from acid or nearly so, as otherwise the characteristic coloring is lost at once.

An advantage of the method is that specimens older than three or four weeks may still be satisfactorily examined. In that event, however, it is necessary to place them in alcohol for some time and then to wash in water so as to be sure that they will actually be moistened by the watery solution of the dye. In old specimens it is also necessary to stain for a much longer time—often for twenty-four hours. Should the preliminary examination in water show that the red cells are stained dark blue or a dark bluish red, which is common with specimens that are old, it is well to dip the specimen into alcohol for a second or two so as to extract some of the dye; it is then again washed in water, dried, and mounted.

In a properly-stained Romanowsky specimen, no matter what modification has been used, the red cells appear red, in overstained or old specimens light gray or light blue. Polychromatophilia and granular degeneration are well shown. The neutrophilic granules are bright red, the eosinophilic granules eosin colored, and the mast-cell granules dark red. The nuclei of the lymphocytes, large mononuclear leucocytes, and myelocytes are bright red, those of the polynuclear leucocytes a bluish violet. In some of the lymphocytes and large mononuclear leucocytes Michaelis' granules will be seen. The blood-

plates are pale blue with red nuclei. The nuclei of the red blood-corpuscles are red. The malarial organisms present a blue body with one or more intensely red nuclear structures varying in size from that of a tiny dot in the youngest forms to a structure which in the microgametocytes fills the entire body of the parasite in the form of a fine reticulum. In the segmenting bodies it will be observed that each segment contains a red nucleus, while the body is blue.

Method of Michaelis and Wolff.[1]—Two solutions are kept on hand: the one is a 1 pro mille aqueous solution of eosin; the other is a methylene-azure solution, which is prepared as follows: 200 c.c. of a 1 per cent. aqueous solution of methylene-blue are boiled for fifteen minutes with 10 c.c. of a decinormal solution of sodium hydrate and then accurately neutralized with 10 c.c. of a decinormal solution of sulphuric acid. Immediately before use, 2 c.c. of the resulting azure blue solution are well mixed with 10 c.c. of the eosin solution. The preparations, which are best fixed by immersion for one hour in absolute alcohol, are placed in watch-crystals in the staining solution, specimen side down, and are allowed to remain for fifteen minutes. They are then washed in water, dried, and mounted as described above (see Nocht's method). The occurrence of a precipitate during the process of staining is disregarded. The various elements of the blood are stained as with Nocht's method.

Method of Giemsa.[2]—Giemsa uses a 1 pro mille aqueous solution of eosin (yellow shade) and a 1 pro mille aqueous solution of pure azure blue (Grübler). Before use, 1 c.c. of the eosin solution is diluted with 10 c.c. of water and treated with 1 c.c. of the azure solution. The specimens are fixed by heat or by immersion in absolute alcohol for one hour, and are then placed in the staining solution, specimen side down, using watch-crystals, for a time varying between ten minutes and one hour, which must be ascertained by trial. They are then washed in water, dried, and mounted as above; the individual elements are stained as with Nocht's method.

Method of Reuter.[3]—Reuter precipitates a solution of methylene-azure in methylene-blue prepared according to Michaelis or Nocht, with a dilute solution of eosin, and makes use of the precipitated eosinate of methylene-blue mixed with eosinate of methylene-azure in alcoholic solution (best methyl alcohol to which some anilin oil is added). A few drops of this solution are added to a watch-crystalful of water, in which the specimens are immersed for one hour. They are then washed in water, dried, and mounted as usual. Precipitates are not formed during the process of staining,

[1] L. Michaelis u. A. Wolff, Virchow's Archiv, 1902, vol. clxvii. p. 151.
[2] Giemsa, Centralbl. f. Bakt., 1902, vol. xxxi.
[3] Reuter, Ibid., 1901, vol. xxv.

but as I have stated above, even the alcoholic solution loses much of its staining power after a few weeks.

The dye can be procured from Grübler already prepared.

The individual elements of the blood are stained as with Nocht's method (see above).

Leishman's Method.[1]—Leishman also makes use of the isolated eosinate of methylene-blue mixed with eosinate of methylene-azure. He proceeds as follows: Two solutions are prepared: one a 1 pro mille solution of eosin (Grübler's extra B.A.) in distilled water; the other a 1 per cent. solution of medicinal methylene-blue (Grübler), also in distilled water, and alkalinized with sodium carbonate to the extent of 0.5 per cent. This last solution is heated to 65° C. for twelve hours, and is then allowed to stand at the temperature of the room for ten days before using. Equal volumes of the two solutions are mixed in a large open basin and are allowed to stand for from six to twelve hours, the mixture being stirred from time to time with a glass rod. The resulting precipitate is collected on a filter, thoroughly washed with distilled water, dried, and powdered. A 0.15 per cent. solution of the dye in pure methyl alcohol serves as stain and does not deteriorate on keeping. Special fixation is not required. The blood-film is covered with the solution and stained for about one-half minute. Double the amount of distilled water is then added and allowed to mix with the alcoholic solution. After from five to ten minutes the stain is washed off with distilled water, a few drops of water being allowed to rest on the film for a minute. The specimen is next dried (without heat) and can then be examined as usual. The soaking in water for a minute after the staining is important, as it intensifies the Romanowsky stain; it changes the tint of the red corpuscles from a greenish-blue to a transparent pink or greenish color, while the nuclei of the leucocytes are usually a ruby red. The nuclei of nucleated red cells are almost black and the extranuclear portion gray. The blood-plates are a deep ruby red with shaggy margins, frequently showing a pale-blue peripheral zone surrounding the red centre. The body of the malarial parasite stains blue and its chromatin a ruby red. In the case of the tertian parasite Schüffner's dots are well marked in the containing red corpuscles.

Wright's Modification of Leishman's Method.[2]—Wright has simplified Leishman's method in several important particulars, which render the method even more convenient for routine work; he has ascertained, moreover, that any one of the Grübler methylene-blues can be employed for the purpose of obtaining a sufficient quantity of methylene-azure.

The staining fluid is prepared as follows: 1 per cent. of methylene-

[1] Leishman, Brit. Med. Jour., Sept. 21, 1901.
[2] Wright, Jour. Med. Research, 1902, vol. vii.

blue is added to a 1 per cent. aqueous solution of sodium bicarbonate, when the mixture is steamed in an Arnold's steam sterilizer for one hour. On cooling, the solution is poured directly into a large dish or flask and treated, while stirring or shaking, with a sufficient quantity of a 1 pro mille solution of eosin (yellow shade) until the mixture has assumed a purple color and a scum with a metallic lustre forms on the surface. This will require about 500 c.c. of the eosin solution for 100 c.c. of the methylene-blue solution. The resultant precipitate, which contains both eosinate of methylene-blue and eosinate of methylene-azure, is collected on a filter, and without further washing is allowed to dry. When thoroughly dry, an 0.3 per cent. solution in *pure* methyl alcohol is prepared (this is practically a saturated solution). The solution is then filtered and to the filtrate 25 per cent. methyl alcohol is further added so as to dilute the stain somewhat and to lessen the tendency of the dye to become precipitated during the process of staining.

The air-dry blood-films are covered with the stain for one minute; water is then added drop by drop until the staining fluid becomes semitranslucent and a reddish tint becomes visible at the margins, while a scum with a metallic lustre forms on the surface. The amount of water required will vary with the amount of staining fluid on the preparation, but in general it may be said that 8 or 10 drops will suffice if a seven-eighths inch square cover-glass is used. The staining fluid, thus diluted, is allowed to remain on the preparation for two or three minutes, during which time the real staining of the specimen takes place. It is then washed off, when the blood-film will be seen to have a blue or purple color.

The next step is to develop the differential staining of the various elements in the preparation. This is done by washing the preparation in water, preferably distilled water, until the better spread portions of the film appear yellowish or reddish in color. If desired, the process of differentiation may be readily observed by placing the cover-glass, film side uppermost, on a slide, covering it with water, and examining it with the microscope under a low magnifying power. The red blood-corpuscles, which, as before stated, at first have a blue color, will become greenish, then yellowish, and finally orange or pinkish in color, depending upon the depth of the original staining, which varies with the length of time that the diluted staining fluid has been allowed to act, and with the degree of its dilution.

The differentiation by washing in water seems to be essentially a process of decolorization by which some of the blue constituent of the dye is removed, for the water that drains off from the preparation has a blue color. This differentiation or decolorization proceeds slowly, and may require from one to three minutes, depending upon the intensity of the staining and upon the tint sought to be obtained in the red corpuscles.

It is apparent from the above that with a little experience with the method the color of the red corpuscles may be made either orange or pink. When the desired color is obtained in the red corpuscles, the preparation is quickly dried between layers of filter-paper and mounted in balsam. It is important to arrest the decolorization by drying the preparation as soon as the desired tint in the red corpuscles is obtained, for it may be carried too far.

Dried blood-films may be kept for weeks without impairment of their staining properties. Films months old will probably not give good results.

In a suitably stained specimen the red cells are either orange or pink; polychromatophilia and granular degeneration are well shown (the granules blue); the neutrophilic granules are a reddish lilac, the eosinophilic granules eosin colored, the mast-cell granules a dark blue, a dark purple, or even black. The lymphocytes have dark purplish-blue nuclei with robin's egg blue protoplasm, in which the granules described by Michaelis appear dark blue or purplish. The large mononuclear leucocytes present a blue or dark lilac colored nucleus, and in some Michaelis' granules can also be made out. The blood-plates are stained a deep blue or purplish and the malarial organisms are colored as with Nocht's method.

Koch's Method.[1]—With Koch's method the modification of the methylene-blue with an alkali is effected during the process of staining. Three stock solutions are kept on hand: the one is a concentrated aqueous solution of medicinal methylene-blue (Höchst); the other is a 1 per cent. aqueous solution of eosin (Höchst B. A. extra); the third is a 5 per cent. aqueous solution of crystallized sodium carbonate. Before use, the different components are mixed as follows: 1 c.c. of the methylene-blue solution is diluted with 10 c.c. of distilled water; to this 3 drops of the soda solution are added, and, while stirring, as much of the eosin solution as will produce a finely granular precipitate.

The blood-films should be fixed by heat or with alcohol and ether, and are stained from five to ten minutes, when they are differentiated in distilled water (about 10 c.c.) to which an oeseful of acetic acid has been added. The differentiation is carried to a point where the eosin tone becomes apparent. The specimens are then rapidly washed off, dried, and mounted as usual.

The various elements will be stained as above.

Ewing's Method.[2]—Ewing makes use of a neutralized solution of Unna's polychrome methylene-blue, which is itself rich in methylene-azure, and which he then combines with eosin and a small amount of methylene-blue. His method in detail is the following:

A neutral solution of Unna's polychrome methylene-blue is pre-

[1] Koch, Deutsch. med. Woch., 1900.
[2] Ewing, Clinical Pathology of the Blood, loc. cit.

pared by adding dilute acetic acid (2–3 per cent. solution) to the commercial polychrome methylene-blue solution (Grübler), until the latter no longer presents an alkaline reaction. As a general rule 5 drops of a 3 per cent. solution of the acid are sufficient for 1 ounce of the commercial liquid dye. The reaction is tested with red litmus-paper, taking note of the color immediately above the zone which comes in contact with the stain.

In addition to the above solution Ewing uses a 1 per cent. aqueous solution of Grübler's methylene-blue, at least a week old, and a 1 per cent. aqueous solution of Grübler's eosin (yellow shade).

The staining reagent is then prepared by adding 4 drops of the eosin, 6 drops of the polychrome methylene-blue, and 2 drops of the ordinary methylene-blue solution to 10 c.c. of distilled water and mixing well. The blood specimens are fixed in alcohol or by heat and are immersed in the stain, specimen side down, for one or two hours, or if necessary even longer; they will not overstain in twenty-four hours. They are then washed in water, dried, and mounted as usual. The different elements of the blood will be stained as with Nocht's method.

Ziemann's Method.[1]—This is warmly recommended by Grawitz as a routine laboratory method. Two stock solutions are employed, the one a 1 pro mille aqueous solution of eosin, the other a 1 per cent. aqueous solution of methylene-blue containing 2.5 grammes of borax in 100 c.c. Before use, the two solutions are mixed in the proportion of 4 parts of the eosin to 1 of the methylene-blue solution and poured over the fixed blood-smears lying face downward in watch-crystals with concave bottom. After five minutes the specimens are washed in water, the iridescent film which forms being thus removed. At this stage the preparations are colored an intense bluish violet, which changes to a reddish tint when they are immersed for a moment or two in *very* dilute acetic acid solution. They are then again washed in water, dried, and mounted. The various elements of the blood are stained as already described (see Nocht's method).

Ehrlich's Eosin-methylene-blue-methylal Solution.[2]—In this method the neutral dye which is formed from the eosin and methylene-blue is held in solution with methylal (methylendimethylether). The reagent consists of 10 c.c. of a 1 per cent. aqueous solution of eosin, to which 8 c.c. of methylal and 10 c.c. of a saturated aqueous solution of medicinal methylene-blue are added. The mixture is ready for use at once, and furnishes good results; it is very unstable, however, and should be freshly prepared whenever it is needed. It is necessary that the specimens should be carefully fixed by heat, as a characteristic coloring is otherwise not obtained. The preparations

[1] As described by Grawitz, Pathol. d. Blutes, 2d ed.
[2] Ehrlich-Lazarus, Die Anaemie, 1898.

are stained for one or two minutes. The eosinophilic granules are stained red, the mast-cell granules a pure blue, and the neutrophilic granules a purplish red.

In my experience the method presents no advantages over Jenner's method and is decidedly more complicated.

Michaelis' Eosin-methylene-blue-acetone Solution.[1]—The neutral dye resulting from the interaction between the eosin and the methylene-blue is here held in solution by the aid of acetone. Like the preceding method, it has no advantages over Jenner's method, and is decidedly more complicated. Two solutions are prepared, viz., one containing 20 c.c. of a 1 per cent. aqueous solution of chemically pure methylene-blue with an equal amount of absolute alcohol; the other is composed of 12 c.c. of a 1 per cent. aqueous solution of chemically pure eosin and 28 c.c. of acetone. These two solutions are kept in separate bottles and are mixed in equal proportions immediately before use. The mixture is placed in a watch-crystal and is covered at once. The blood-films should be fixed by heat or by immersion in absolute alcohol for from one to twenty-four hours, and are then placed *in* the stain, face downward, for from one-half to ten minutes, the time varying with the individual preparations. The staining should be interrupted as soon as the blue color which first appears has turned to red, as otherwise the nuclei of the leucocytes will be decolorized. If the leucocytes are materially increased, it is best to stop even before this point is reached. If, on the other hand, the blue stain has acted too energetically, the specimens are stained a little longer. The preparations are finally rinsed in water, thoroughly dried, and mounted as usual. The various elements of the blood in a successfully stained specimen will be found colored as with Jenner's method.

Strauss and Rohnstein's Method.[2]—The preparations are first stained with a solution of rubeosin and then counterstained with methylene-blue, which stains in an elective manner. Strauss and Rohnstein suggest the method for routine work. The technique in detail is the following: The blood-smears are fixed by heat, alcohol-ether, or formalin, and after drying are stained for three minutes in a mixture of the following solutions (3 parts of A to 1 part of B):

A

Eosin (yellow shade, Grübler)	0.5 gramme
Absolute alcohol	80.0 grammes
Water	20.0 "

B

Rubin (fuchsin, Grübler)	0.5 gramme
Absolute alcohol	80.0 grammes
Water	20.0 "

[1] Michaelis, Deutsch. med. Woch., 1899, p. 490, and 1901.

[2] H. Strauss u. R. Rohnstein, Die Blutzusammensetzung b. d. verschiedenen Anaemien, Berlin, 1901, Hirschwald.

The specimens are then washed in water, stained in a $\frac{1}{2}$ to $\frac{1}{3}$ per cent. aqueous solution of the methylene-blue (which if possible should be at least a couple of weeks old), again washed in water, dried, and mounted.

The red cells are colored a brick red, the nuclei and all basophilic elements marine-blue, the neutrophilic granules a bluish-brownish violet, and the eosinophilic granules a bright red.

Strauss and Rohnstein state that overexposure in one or the other solution does not matter very materially, and that the AB mixture keeps for a long time.

Special Staining Methods.—Staining with Eosin.—The blood-smears are best fixed by heat or by immersion in absolute alcohol and are then stained for about a minute with an 0.25 to 0.5 per cent. alcoholic (70 per cent.) solution of eosin, or for ten to twenty minutes with an 0.1 to 0.5 per cent. aqueous solution of the dye. They are then washed in water, dried, and mounted as usual. The red corpuscles and the eosinophilic granules are stained a bright red, and the protoplasm of the leucocytes a faint red.

The stain is used to demonstrate the presence of oxyphilic material.

Staining with Ehrlich's Triglycerin Mixture.[1]—This is composed of 2 grammes each of eosin, aurantia, and indulin in 30 grammes of glycerin. These constituents are brought into solution by keeping the mixture in the warm chamber (37° to 40° C.) for about one week.

The specimens must be well fixed, an exposure to a temperature of about 110° C. for at least two hours being best. They are then allowed to remain upon the stain for from sixteen to twenty-four hours, when they are rinsed in water, dried, and mounted as usual. The red corpuscles are colored orange, the bodies of the leucocytes a dirty gray, with dark nuclei, and the eosinophilic granules a bright red. If, however, the specimens are fixed too intensely, the eosinophilic granules take the aurantia and are thus colored orange.

The stain is used for the demonstration of oxyphilia and its degree.

Ehrlich's Neutral Mixture.[2]—This consists of 5 volumes of a saturated aqueous solution of acid fuchsin, to which 1 volume of a saturated aqueous solution of methylene-blue is slowly added while stirring. The mixture is treated with 5 volumes of distilled water and filtered after having stood for several days. The specimens are stained for from five to twenty minutes. Only a slight degree of fixation is necessary.

The red corpuscles are stained the color of fuchsin, their nuclei as well as those of the leucocytes are black or a light lilac, the eosinophilic granules red, and the neutrophilic granules violet.

The stain is used for the demonstration of neutrophilic material.

[1] Ehrlich-Lazarus, loc. cit. [2] Ibid.

Basic Double Staining.—Ehrlich's Methyl-green-fuchsin Mixture.[1]—A saturated aqueous solution of methyl-green is treated with a small amount of an alcoholic solution of fuchsin. After brief fixation the specimens are stained for five minutes. The nuclei appear green, the red corpuscles red, and the protoplasm of the lymphocytes the color of fuchsin. The stain is especially serviceable for demonstration purposes in cases of lymphatic leukæmia.

Instead of methyl-green, one can also use iodine-green, but not malachite-green or chromgreen ; instead of the fuchsin, para- (rosanilin) rubin, saffranin, acridin-red, neutral red, or best of all pyronin may be employed.

Pappenheim's Method.[2]—Pappenheim suggests a stain which is composed of a concentrated aqueous solution of methyl-green, to which pyronin is added until the solution just begins to turn blue viz., about 1 part of pyronin for 3 to 4 parts of methyl-green. Stained in this manner the basophilic protoplasm of the lymphocytes is colored a fine dark carmine red, while the protoplasm of all other cells is stained a more or less pale brownish or reddish-yellow, or remains colorless. Pappenheim regards this stain as essentially specific for the lymphocytes, but admits that it also stains in a similar manner the young erythroblasts that are poor in hæmoglobin. The difference can be recognized from the character of the nuclei and the fact that the margin of the lymphocytes very commonly appears shaggy, while that of the erythroblasts is smooth and homogeneous.

Special Mast-cell Stains.—Ehrlich's Dahlia.[3]—The staining fluid consists of 100 c.c. of distilled water to which 50 c.c. of a saturated alcoholic (absolute) solution of dahlia are added. On clearing, this solution is further treated with 10 to 12.5 c.c. of glacial acetic acid. The blood-smears should be fixed by heat or absolute alcohol, and are then stained for from five to ten minutes. With the exception of bacteria, only the mast-cells are stained, while the neutrophilic leucocytes are only faintly tinged.

Pure methyl-green does not stain the mast-cell granules, but the impure products which are contaminated with methyl-violet give good results. This is owing to the fact that methyl-green is a pure nuclear dye.

Westphal's Method.[4]—This furnishes very handsome pictures. 100 c.c. of a carmine solution are prepared by dissolving 2 grammes of pure carmine in 200 c.c. of distilled water with 5 grammes of alum ; the mixture is boiled for fifteen minutes, filtered, and treated with 1 gramme of carbolic acid. This solution is then mixed with 100 c.c. of glycerin, 100 c.c. of a concentrated solution of dahlia in

[1] Ehrlich-Lazarus, loc. cit.
[2] Pappenheim, Virchow's Archiv, 1899, vol. clvii.
[3] Ehrlich-Lazarus, loc. cit.
[4] Westphal, Inaug. Diss., Berlin, 1880.

absolute alcohol, and 20 c.c. of glacial acetic acid. The specimens are stained for about twenty-four hours. The mast-cell granules and any bacteria that may be present are colored a bluish violet, while all nuclei are colored red.

Methylene-azure.—Methylene-azure is also well adapted for the demonstration of mast-cells ; it may be used in any one of the modifications described under the heading of the Romanowsky stain. The granules are stained metachromatically (see page 80).

Methods of Chenzinsky-Plehn.—The methods of Chenzinsky and Plehn,[1] which were formerly extensively used in the study of malarial blood, are now rarely employed. The staining reagents are eosin-methylene-blue mixtures, in which the resulting neutral dye, the eosinate of methylene-blue, is held in solution by an excess of the basic component. Both methods are here described, as they may possibly still be used when other reagents are not available.

Chenzinsky Method.—The reagent consists of 40 c.c. of a concentrated aqueous solution of methylene-blue, to which 20 c.c. of a 0.5 per cent. solution of eosin in 70 per cent. alcohol and 40 c.c. of distilled water have been added. The solution keeps fairly well, but should always be filtered before using. A slight degree of fixation only is necessary—five minutes in absolute alcohol. The specimens are stained for from six to twenty-four hours in air-tight watch-crystals at a temperature of 37° to 40° C.

The red corpuscles and eosinophilic granules are stained a bright red, the nuclei and mast-cell granules a dark blue, the bodies of the malarial organisms a light sky blue. The neutrophilic granulation remains unstained.

Plehn's Method.—The stain consists of 60 c.c. of a concentrated aqueous solution of methylene-blue, 20 c.c. of a 0.5 per cent. solution of eosin in 75 per cent. alcohol, 40 c.c. of distilled water, and 12 drops of a 20 per cent. solution of caustic alkali.

The specimens are fixed by heat and stained as with Chenzinsky's method ; the coloring is the same.

Futcher's Carbol-thionin.—This method, which was formerly extensively employed in the study of malarial blood, is no longer in common use. It gives good results, but unfortunately the specimens soon fade out.

The air-dried films are fixed for one minute in a 0.25 per cent. solution of formalin in 95 per cent. alcohol. But as it is important that this solution should be made up fresh for each examination, it is more convenient to keep a 10 per cent. aqueous solution of formalin on hand, and to add 4 or 5 drops of this to 10 c.c. of 95 per cent. alcohol just before using. The specimens are then rinsed in water, dried between filter-paper, and stained for from ten to fifteen seconds with a carbolated solution of thionin. This is prepared by

[1] Plehn, Aetiol. u. klin. Malariastud., Berlin, 1890, Hirschwald.

adding 20 c.c. of a saturated solution of thionin in 50 per cent. alcohol to 100 c.c. of a 2 per cent. solution of carbolic acid. The thionin carbolate thus formed constitutes the active staining principle. After washing off the excess of stain the preparations are dried with filter-paper and mounted as usual. Thus prepared, the malarial parasites appear as reddish-violet bodies and are readily seen. The method is of special value in staining the ring-shaped bodies of the æstivo-autumnal infection, which are difficult to recognize in unstained specimens, and usually do not stain well with eosin and methylene-blue.

Staining with Ehrlich's Hæmatoxylin-eosin, or Orange-G Solution.—The solution is prepared by dissolving 2 grammes of hæmatoxylin in a mixture of 100 grammes each of distilled water, alcohol, and glycerin. To this solution 10 grammes of glacial acetic acid and an excess of alum are added. After exposure to the sunlight for from four to six weeks about 0.5 gramme of eosin or orange-G is added.

The specimens are fixed in absolute alcohol, or by heat (a brief exposure only is necessary). They are then left in the stain, in the sunlight, for from one-half to two hours, when they are thoroughly washed in water, dried, and mounted.

The red corpuscles and eosinophilic granules are colored a bright red, the nuclei of normoblasts and megaloblasts a deep black, the bodies of the leucocytes a light lilac, and their nuclei a dark lilac. The bodies of the lymphocytes, however, are scarcely stained at all, while their nuclei appear only a shade lighter than those of the nucleated red corpuscles.

Demonstration of Iodophilia.—Cover-glass specimens are prepared as usual; after drying in the air they are placed in a small jar containing a few crystals of iodine. After several minutes the films assume a dark-brown color, when they are mounted in a drop of a saturated solution of lævulose and examined with an oil-immersion lens. The red corpuscles are stained the color of iodine, while the leucocytes are almost colorless. All glycogen granules, whether contained in leucocytes or free in the blood, are stained a distinct mahogany.

This method furnishes better results than the older method of staining with a solution composed of 1 gramme of iodine and 3 grammes of potassium iodide in 100 grammes of a concentrated solution of mucilage (1 part of Lugol's solution to 100 parts of a thick mucilage).[1]

In place of the method just described, Barfurth's procedure may also be employed. In this case the solution is prepared by mixing 1 part of Lugol's solution with 2 parts of glycerin. The preparations do not keep well, however, as the glycerin gradually extracts the iodine.

[1] Ehrlich-Lazarus, Die Anaemie, loc. cit.

Distribution of the Alkali in the Blood.

A very good idea of the distribution of the alkali in the blood may be formed by making use of the following method, suggested by Ehrlich: a drop of blood is carefully spread between two cover-glasses, when the air-dried specimens are immediately placed in a watch-crystal, containing a solution of the free staining acid of erythrosin in chloroform. In a few minutes the specimens have assumed a bright-red color, when they are transferred for a minute or two into a crystal containing chloroform. While still moist they are then imbedded in Canada balsam. Prepared in this manner, the alkaline elements of the blood are colored red. The plasma presents a distinctly red color, while the red corpuscles have not taken up the stain. The protoplasm of the leucocytes and especially of the lymphocytes, as also the plaques, the fibrin-filaments, and the bits of protoplasm derived from the leucocytes are all stained a deep red, while the nuclei of the leucocytes remain colorless. If malarial organisms are present, these are likewise stained.

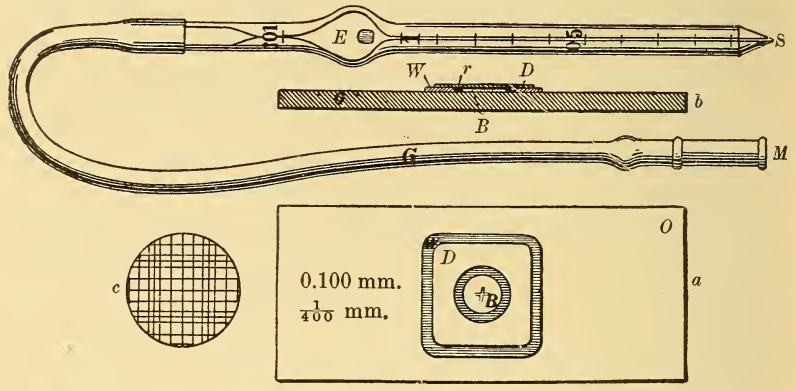
In order to prepare the stain, a saturated aqueous solution of erythrosin (tetra-iodo-fluorescëin) is acidified with dilute hydrochloric acid, and the staining acid, which is thus precipitated, collected on a filter, after having been washed with distilled water. The precipitate is dissolved in chloroform, to which it imparts an orange color. This solution is employed for staining. In every case care should be had that the glass utensils which are used are freed from adherent alkali by washing with concentrated acids and then with distilled water.

Enumeration of the Corpuscles of the Blood.

Method of Thoma-Zeiss.—Of the various instruments devised for the enumeration of the corpuscles of the blood, that of Thoma-Zeiss is almost exclusively used in the United States. Its essential parts are two diluting pipettes and a counting-chamber. One pipette permits of a dilution of the blood in the proportion of 1 : 100 to 1 : 1000, and is generally used in the enumeration of the red corpuscles; the other pipette, which has been devised for counting the white cells, will allow a dilution of 1 : 10 to 1 : 100. Each pipette has a small bulb blown on its stem, which contains a glass bead to facilitate the mixing of the blood and the diluting fluid. The principle underlying the construction of the counting-chamber will be seen in the accompanying illustration (Fig. 17). It is essentially a large slide to which the glass plate D is cemented. This, it will be noted, has its central area cut out, and there is cemented to the centre of this area a round platelet of glass, the surface of which is exactly 0.1 mm. below the surface of D. If then a cover-glass is placed upon D, there will be an underlying chamber, B, which is filled by

a droplet of the diluted blood. To facilitate enumeration of the individual corpuscles, a portion of the central platform is ruled into sets of small squares, each of which has an area of exactly $\frac{1}{400}$ square mm. As the depth of the chamber is 0.1 mm., each square represents the base of a cube, the contents of which will be $\frac{1}{400} \times \frac{1}{10}$—that is, $\frac{1}{4000}$ cbmm. In counting the red cells the object is to ascertain the average number of cells in a small square by counting a large number of squares, when the corresponding number in the undiluted blood is ascertained by multiplying by 4000 and the degree

FIG. 17.



Thoma-Zeiss blood-counting apparatus.

of dilution. Similar considerations underlie the determination of the number of leucocytes per unit of blood—*i. e.*, per cbmm. (see below).

In the accompanying diagram (Plate IX.) I have represented the Türk ruling which is especially convenient. It is a modification of the Zappert ruling and well adapted for counting the leucocytes and red corpuscles in one and the same specimen. It is made up of large and small squares, which for convenience' sake I have represented partly in black and partly in red.

Special Technique.—Enumeration of the Red Cells.—To secure the necessary amount of blood, it is more convenient to puncture the finger than to obtain the blood from the ear, as the hand of the operator can be steadied against the finger of the patient. The finger is washed with soap and water, then with alcohol and dried. The puncture should be sufficiently deep that a large drop of blood can be obtained without special pressure. If there is reason to believe that the patient is not very anæmic, the capillary pipette is filled to the mark 0.5, which will furnish a dilution of 1 : 200; otherwise the blood is drawn to the mark 1, which will give a dilution of 1 : 100. In filling the pipette the tube is held between the thumb and forefinger of the right hand, while the finger of the patient

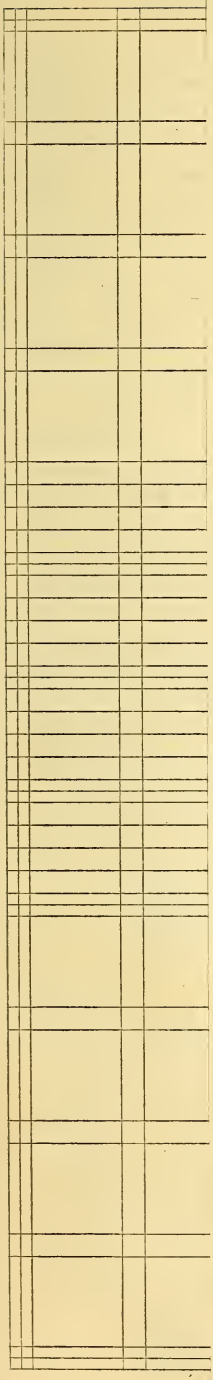
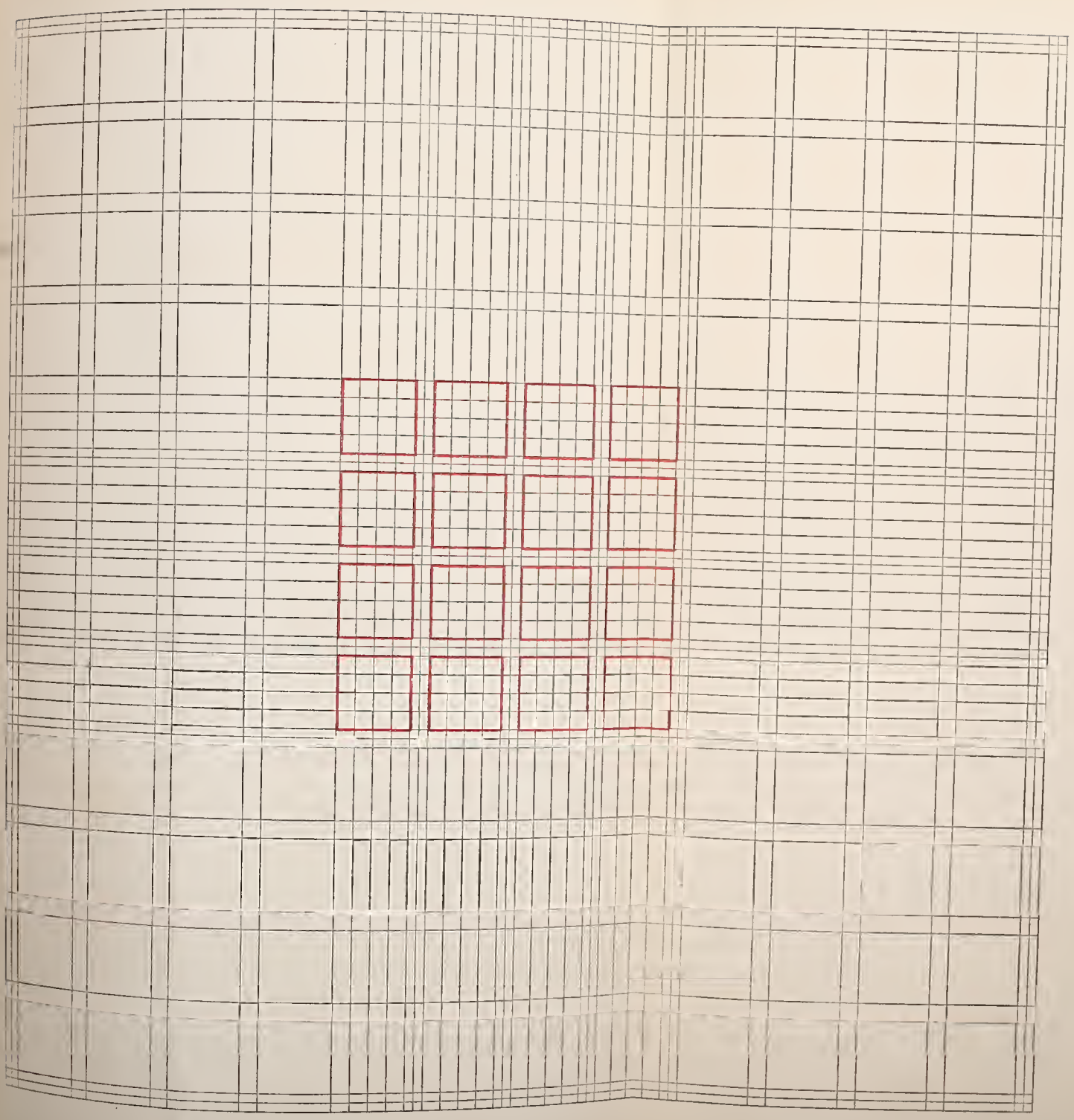


PLATE IX.



Türk's Counting Chamber.

There are in all 144 large squares, for counting the leucocytes; the central block of 16, ruled in red, is used in counting the red cells.

is held with the fingers of the left hand; at the same time the right hand is steadied by placing the little finger or ring finger of the right hand against the patient's finger from which the blood is being drawn.

As soon as the requisite amount of blood has been obtained, the end of the pipette is rapidly but carefully wiped of adherent blood with the finger and then immediately plunged into the diluting fluid, which should be ready at hand. This is drawn up to the mark 101, while the pipette is gently rotated between two fingers. When the 101 mark has been reached, the blood is intimately mixed with the diluting fluid by further rotation. The contents of the capillary tube are now expelled, the remaining mixture representing a 1 : 100 or 1 : 200 dilution of the blood, as the case may be.

A few *precautions* during this step of the process should be observed: It is essential that the degree of dilution should be accurate, and it is therefore a prime requisite that the blood column should just reach the 0.5 or the 1 mark, as the case may be, and that similarly the 101 line is not exceeded. If the blood has been drawn too far, as often happens with beginners, the tube must be cleansed and another attempt made. In such a case the diluting fluid is drawn up exactly as though no accident had happened, so as to prevent the column of blood from coagulating in the capillary tube. In any event only a few seconds should be allowed to elapse from the time that the tube is filled with the blood until the diluting fluid is drawn up. Should air-bubbles enter the blood column, the entire process must, of course, be repeated.

For the purpose of diluting the blood Toison's solution is most commonly used. It has the following composition:

Sodium chloride	1.000 gramme.
Sodium sulphate	8.000 grammes.
Neutral glycerin	30.000 "
Distilled water	160.000 "
Methyl-violet 5 B	0.025 gramme.

The addition of the methyl-violet serves the purpose of coloring the leucocytes, which are thus rendered quite easily visible.

Other solutions which may be employed are the following: physiological salt solution—viz., 0.9 per cent.; a 15–20 per cent. solution of magnesium sulphate; a 5 per cent. solution of sodium sulphate; one of osmic acid (1 : 4000); Lugol's solution (iodine, 1 gramme; potassium iodide, 2 grammes; and water, 300 c.c.); the special reagents of Hayem, Pacini, Petrone, Acquisto, and Marcano, the formulæ of which follow:

Formula of Hayem's fluid:

Mercuric chloride	0.5 gramme.
Sodium sulphate	5.0 grammes.
Sodium chloride	2.0 "
Distilled water	200.0 "

Formula of Pacini's fluid:

Mercuric chloride	2.0 grammes.
Sodium chloride	4.0 "
Glycerin	26.0 "
Distilled water	226.0 "

Formula of Petrone's fluid:

Mercuric chloride	1.0 gramme.
Sodium chloride	7.0 grammes.
Distilled water	100.0 "

Of this solution 10 drops are diluted with 90 drops of distilled water; the resultant solution is used as the diluent.

Formula of Acquisto's solution:

An 0.5 per cent. solution of chromic acid	1 part.
Sulphopicric acid	1 "
A 1 pro mille solution of mercuric chloride	1 "
A mixture of absolute alcohol and $\frac{1}{3}$ glacial acetic acid	1 "

The resultant solution is diluted with 2 parts of water before use.

Formula of Marcano's reagent:

Formol	1.0 gramme.
Sodium chloride	1.0 "
Distilled water	85–100 grammes.

Before use, the liquid is diluted with 2 volumes of distilled water.

After the pipette has been filled in the manner indicated and the contents of the capillary tube have been expelled, a drop of the diluted blood is placed on the platform of the counter and is covered with one of the specially ground cover-glasses which accompany the instrument. This step of the process requires great care and some experience. It is essential that the apparatus should be perfectly clean and dry, that no air-bubbles enter, that the entire platform is covered, and that none of the liquid flows over between the cover-glasses and the plate D. It is necessary, moreover, that the corpuscles should be evenly distributed over the platform, which is readily ascertained by a superficial examination with a low power after the corpuscles have settled. In a well-prepared specimen Newton's colored rings will appear between the cover-glass and the plate D, if moderate pressure is exerted upon the cover, especially if the surface of D has been slightly moistened by breathing upon it *very gently* before the cover-glass is applied. Personally I advise that the drop should be of such size that it exactly covers the platform after the cover is adjusted, and that no portion of the liquid shall project over or flow into the moat. Otherwise currents are set up which are especially apt to produce an irregular distribution of the cells. The cover-glass must be rapidly adjusted and firmly pressed into position. If then a rapid survey shows that the specimen is satisfactory, the count is begun. Using a Leitz No. 7 or

a Bausch & Lomb $\frac{1}{6}$,[1] the left upper large square, composed of 16 small squares, is brought into the field (see Plate IX.). In this the red cells are counted in rows of 4 horizontal small squares. The slide is then moved to the adjoining large square. In doing so it will be noted that every large square is separated from its neighbor both horizontally and vertically by a row of small cells traversed by a mesially placed line, which serves as a guide to the next large square. As a general rule it will be found more convenient to ignore these intermediary squares and to take account only of the large ones. The results are marked as in the first square, and so on throughout the entire set of 16 large squares, cells that lie on the boundary-line of a square being included when they touch the upper and left border, while they are excluded when they are found on the right and lower lines. The final calculation is made as follows: All the red cells counted are summed up and divided by the corresponding number of small squares, so as to ascertain the average number of red cells for 1 *small* square, the cubic contents of which, as we have seen, is $\frac{1}{4000}$ cbmm. To ascertain the number of red cells in 1 cbmm. of *diluted* blood, this number is hence multiplied by 4000, and the result by the degree of dilution, which will give the number of red cells in 1 cbmm. of *un*diluted blood.

Example.—Dilution, 200 times; number of red cells counted in the 16 large squares—*i. e.*, $16 \times 16 = 256$ small squares—was 1536; this means 6 cells for each small square or $\frac{1}{4000}$ cbmm.; hence in 1 cbmm. of diluted blood $6 \times 4000 = 24{,}000$ cells, and in 1 cbmm. of undiluted blood $24{,}000 \times 200 = 4{,}800{,}000$ red corpuscles.

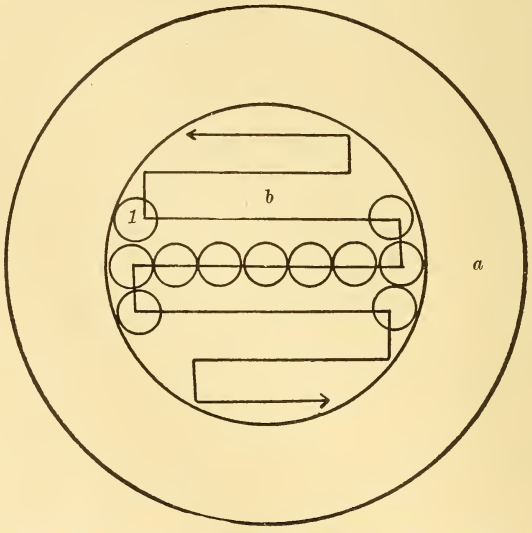
Enumeration of the Leucocytes.—First Method.—Enumeration of the leucocytes has been much facilitated by the introduction of such counting chambers as that of Türk, described above and pictured in Plate IX. If such a counter is available, a special mixing pipette, as I have described above, which permits of a dilution of 1 : 10, is not necessary. It is nevertheless convenient to have two pipettes of the same calibre. For counting the white cells, a dilution of 1 : 100 is then chosen, while the red cells may be diluted to 1 : 200 when there is reason to believe that their number is not materially diminished. To count the red cells in a 1 : 100 dilution, when they are present in approximately normal numbers, is rather taxing to the eyes. Such a pipette is decidedly less difficult to handle than one in which still lower grades of dilution can be secured. Many of the instruments now sent out by Leitz contain two "red" pipettes together with the Türk chamber. The ruling of this chamber is pictured in the accompanying plate (IX.). It will be observed that there are, in all, 144 large squares, the central block of 16 of which is ruled into the smaller squares used in counting the red

[1] To focus through the thick cover Bausch & Lomb have constructed a $\frac{1}{6}$ with a specially long working distance.

cells. The vertical and horizontal lines which serve for the ruling of the small squares have been extended to the margin of the outer ruling, but are ignored in the enumeration of the leucocytes. Using this chamber, the blood is diluted 100 times with Toison's fluid, which colors the leucocytes blue; a drop is then mounted as in counting the red cells. Starting with the left upper square, all leucocytes are counted in the entire set of 144 large squares, including the 16 central squares, which are subruled into blocks of 16 small squares each. Leucocytes that lie in the guiding spaces between the large squares are ignored, and as in counting the red cells corpuscles on the upper and left border-lines of a square are counted in, while those on the right and lower lines are counted out. From the total number the average for 1 large square is calculated. As each large square represents $\frac{1}{250}$ cbmm., the number of leucocytes in 1 cbmm. of non-diluted blood is ascertained by multiplying the average figure by 250 and the product by the degree of dilution—*i. e.*, 100.

Example.—50 leucocytes are seen in the 144 large squares; the average for 1 would be 0.34; in 1 cbmm. of diluted blood there would be $250 \times 0.34 = 85$, and in 1 cbmm. of undiluted blood $85 \times 100 = 8500$ leucocytes.

FIG. 18.



Schema of circles for counting leucocytes: *a*, moat surrounding central platform, *b*, of counter; 1, starting-point.

SECOND METHOD.—If a counting-chamber with a Türk ruling, or one of its modifications, is not available, but if a mechanical stage is at hand, the leucocytes can also be conveniently counted with the old pattern counter, using a red pipette for diluting the

blood. A drop of the diluted blood is mounted as usual. With the mechanical stage a field corresponding to the position of 1 in the accompanying diagram (Fig. 18) is then selected as the starting-point. The presence or absence of leucocytes is noted and the field changed, so that an adjoining circle is brought into view, and so on. In this manner at least 100 circles are gone over, using a corpuscle to the side or above or below as a guide to the next field. The total number of leucocytes is noted and the average for one circle calculated. If the cubic contents corresponding to each circle are known, the calculation of the number of leucocytes in 1 cbmm. of blood becomes a simple matter. The determination of the cubic contents corresponding to a circle is made as follows: noting the number of the eyepiece and the objective, the diameter of the field of vision is measured with a stage micrometer, or with the aid of the rulings of an ordinary Thoma-Zeiss counter, bearing in mind in the latter case that the distance between two vertical lines is $\frac{1}{20}$ mm. The area of the circle, according to geometrical law, will then be equivalent to $\pi\rho^2$, in which π is a constant factor—*i. e.*, 3.1416; and ρ the radius, from which the corresponding cubic contents are calculated by multiplying the result by 0.1—*i. e.*, the depth of the counting-chamber. The resultant value, which should be ascertained for every instrument separately, will, of course, be constant for the system of lenses and the counting-chamber used. With a Bausch & Lomb $\frac{1}{6}$ (long-working distance), the 1 inch eyepiece, and 160 mm. tube length, the cubic contents of the field are 0.009 cbmm.

Example.—The blood was diluted 100 times. In 100 fields 50 leucocytes were noted—*i. e.*, 0.5 for 1 field, or for 0.009 cbmm.; in 1 cbmm. of diluted blood there would hence be 0.5 divided by $0.009 = 55.5$, and for 1 cbmm. of undiluted blood $55.5 \times 100 = 5550$ leucocytes.

Third Method.—If for any reason the 1 : 10 pipette is to be used in counting the leucocytes, it is necessary to choose a diluent which will destroy the red cells, as otherwise they would be relatively so numerous as to obscure the leucocytes altogether. For this purpose an 0.3 per cent. solution of acetic acid is used, which is tinged with gentian-violet and to which a small amount of toluol has been added as a preservative. With this diluting fluid the red cells will be rendered invisible. The leucocytes can then be counted with the ordinary Thoma counting-chamber, or with that of Türk; in the calculation the degree of dilution must, of course, be borne in mind.

Cleaning of the Apparatus.—After use, the apparatus must be carefully cleansed. The pipette is washed out with the diluting fluid, then with water, next with absolute alcohol, and finally with ether. The washing will be facilitated by slipping the rubber tube over the long arm of the pipette and blowing the contents of the bulb out of the short arm. In laboratories which are equipped with a suction-

pump this may be conveniently employed; the entire process then occupies only two or three minutes.

The counting-chamber is washed with water only; alcohol and ether dissolve the substance with which the platform is cemented to the slide.

Differential Enumeration of the Leucocytes.—The differential enumeration of the leucocytes is usually made in dried and stained specimens. A mechanical stage is a great convenience, but not a necessity. The idea is to go over a large number of cells, for ordinary purposes not less than 500–600, to classify these, and finally to calculate the percentages. The cells are charted as shown below:

S. M. (small mononuclear leucocytes): 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 = 45

L. M. and T. F. (large mononuclear leucocytes and transition-forms): 𝍸 𝍸 𝍸 = 15

P. (polynuclear neutrophiles): 𝍸 = 155

E. (eosinophiles): 𝍸 = 5

M. (mast-cells): // = 2

222

Result: Total number of cells counted, 222, of which:

small monos., $\frac{45 \times 100}{222}$ = 20.2 per cent.

large monos., $\frac{15 \times 100}{222}$ = 6.7 " "

polys., $\frac{155 \times 100}{222}$ = 69.8 " "

eosins., $\frac{5 \times 100}{222}$ = 2.2 " "

mast., $\frac{2 \times 100}{222}$ = 0.9 " "

While making a differential count it is always well to keep note of the time, as it is often possible in this way to form a fair idea of the *actual* number of the leucocytes without an absolute count. This, of course, requires a certain amount of experience in the preparation of the smears, which should be uniformly of nearly the same thickness. After one has then learned by control how many leucocytes in a blood-smear, observed within a certain length of time, may be considered as normal, it is not difficult to judge the grade of a hyperleucocytosis by the increase in number noted within the same length of time. Every one must here work out his personal equation. A general idea of the degree of increase can, of course, be formed by examining the specimen with a low power—a Bausch & Lomb $\frac{2}{3}$, for example—but in the manner indicated one gets a numerical expression which is at times quite helpful.

Enumeration of the Plaques.—For this purpose the method of Brodie and Russel has been especially advocated. The method is an indirect one. First, the red corpuscles are counted in the usual manner. A drop of the staining fluid, composed of equal parts of a 2 per cent. solution of common salt and a saturated solution of dahlia in glycerin, is then placed upon the finger, when this is punctured through the drop and the blood is allowed to mix with the reagent. In this mixture the ratio between the plaques and the red corpuscles is ascertained, and the total number of plaques contained in 1 cbmm. of blood determined by calculation. The plaques are stained the color of dahlia and can readily be counted. Rapid work is essential, as the staining fluid soon attacks the red corpuscles.

Ehrlich suggests enumeration of the plaques in air-dried specimens which have been stained with acid erythrosin. Owing to the relatively large amount of alkali which the plaques contain, they are stained an intense red with this reagent (page 139).

Rosin proposes that the air-dried specimens be fixed for twenty minutes by exposure to the vapors of osmic acid, and then stained in a concentrated aqueous solution of methylene-blue.

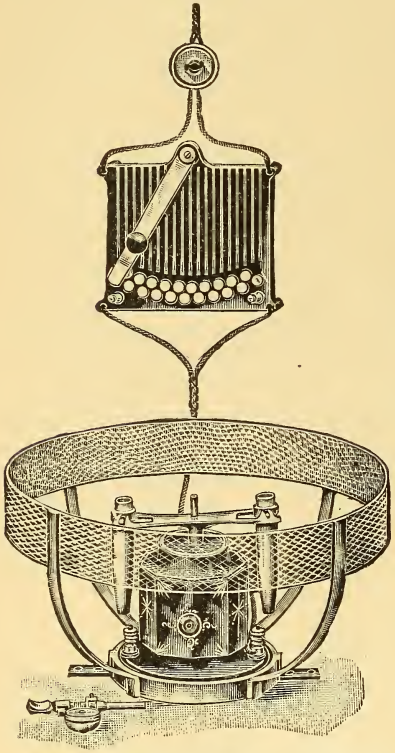
These more or less complicated procedures, however, are not necessary as it is quite possible to count the plaques with the Thoma-Zeiss counter if Petrone's or Acquisto's diluent is employed (which see). The latter especially is useful in the modification suggested by Dotto, according to which 1 drop of a saturated aqueous solution of methyl-green is added to every 5 c.c. of the reagent. In this way the plaques are colored a light green. The mixing pipette should be washed out with the diluent before the blood is drawn up.

The Hæmatocrit.

The use of the hæmatocrit for counting the red blood-corpuscles has been repeatedly advocated, but has not met with much favor. The method is unfortunately inapplicable whenever there is any material variation in the size and form of the red corpuscles and whenever the number of the leucocytes is greatly increased. This means that the method cannot be employed in the majority of cases in which we are especially interested in the blood count. If, however, it is desired to ascertain the volume of the red corpuscles in relation to the amount of plasma, the instrument will furnish satisfactory results. A centrifuge run by electricity is practically a necessity ; in this way alone is it possible to maintain the proper rate and uniformity of speed. Hand centrifuges are, in my experience, totally inadequate for the purpose, and with instruments driven by water-power it is impossible to attain a sufficient rate of speed. An apparatus like the one pictured in the accompanying illustration (Fig. 19) answers the purpose best. It is connected with the street current or with a small battery, a rheostat being

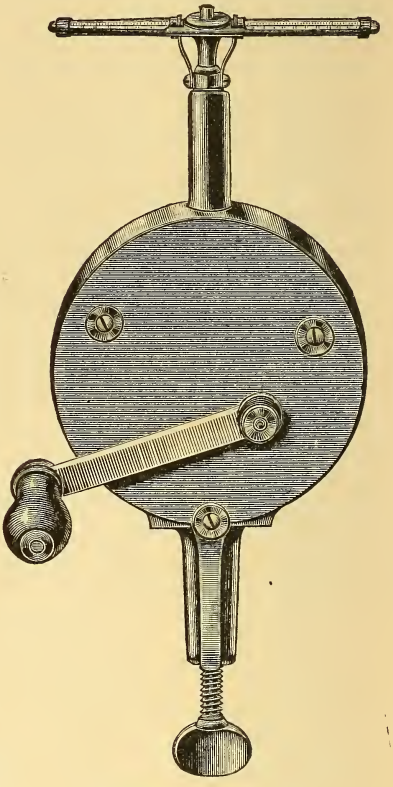
interposed to control the current and the rate of speed. At the same time a speed-indicator can be attached which strikes a bell for every 100 revolutions. For the hæmatocrit a speed of 8000 to 10,000 revolutions per minute is required.

Fig. 19.



Improved electric hæmatocrit, with fender, rheostat, and speed-indicator. The hæmatocrit attachment replaces the urine tubes seen in the revolving armature.

Fig. 20.



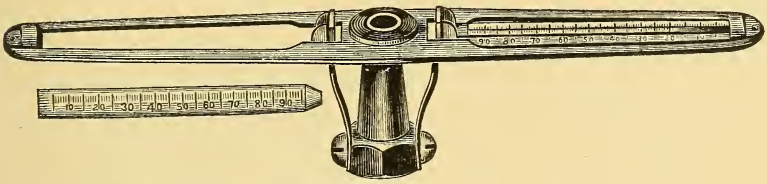
Daland's hæmatokrit.

The hæmatocrit which is almost exclusively used in the United States is that of Daland (Figs. 20, 21, 22). It consists of a metallic frame which carries two glass tubes measuring 50 mm. in length, and 0.5 mm. in diameter. Each tube bears a scale ranging from 0 to 100, the individual divisions of which are rendered more easily visible by a magnifying lens front. In the frame the outer end of each tube fits into a small depression, the bottom of which is covered with thin rubber; the inner ends are held in position by springs. The instrument is screwed to a firm table and is oiled daily when in use.

If the patient is directly available, undiluted blood is used. The finger is washed with soap and water and alcohol, as usual, and is

freely punctured. A small rubber tube is then slipped over the end of one of the hæmatocrit tubes, which is completely filled by suction. The bevelled end of the tube is quickly covered with the finger, which has been previously lubricated with a little vaseline; the rubber tube is disconnected, and the glass tube immediately fixed in the one compartment of the frame. Its mate is rapidly placed on the opposite side and the instrument rotated at a speed of from

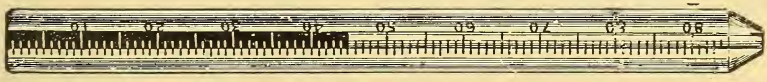
FIG. 21.



Daland's hæmatokrit.

8000 to 10,000 revolutions per minute, for three minutes, when the volume is directly read off. In normal individuals the volume of the red corpuscles is approximately 50 per cent., so that in a given case a proportionate expression of the percentage of corpuscles, as compared with the normal, can be obtained by multiplying the figure on the scale by 2.

FIG. 22.



Daland's hæmatokrit.

If the patient is not directly available, the blood is diluted with an equal volume of a 2.5 per cent. solution of potassium bichromate, as proposed by Daland. As Ewing suggests, this can be done with the pipette which accompanies the Thoma-Zeiss blood-counter. In the case of the red pipette the capillary tube is filled with blood to the mark 1, then a small air-bubble is drawn in, followed by another tube-length of blood. Three or four volumes of blood are obtained in this way and diluted at once with an equal quantity of the bichromate solution. In the case of the white pipette a single tube-length of blood and the diluent is sufficient. Blood and diluent are thoroughly mixed, care being had not to include any air-bubbles. In this form the blood is carried to the laboratory, where both tubes are filled by allowing the drops to flow in from the point of the pipette. To obtain the percentage volume, the resultant figure is in this case, of course, multiplied by 4.

In the case of normal blood it has been ascertained that 1 per cent. by volume, as read off from the scale, corresponds to almost

100,000 red corpuscles per cbmm.; to obtain the total number of red cells per cbmm., it is hence only necessary to add five ciphers to the percentage indicated on the scale.

Example.—Undiluted blood was used; the reading on the scale was 45. The volume per cent. of the red corpuscles would hence be 90, and the number of red cells per cbmm. 4,500,000.

But, as I have pointed out, this calculation presupposes that the size and form of the red cells are practically normal, and that the leucocytes are not materially increased.

With normal blood the leucocytes appear only as a narrow, indistinct milky band at the central end of the column of red cells, which with a material increase of the leucocytes becomes more marked and reaches its greatest extent in well-marked cases of leukæmia.

Aspelin has recently suggested that with a suitable modification of the Daland apparatus quite accurate leucocyte counts can be obtained by centrifugation; but bearing in mind the variations in the size of the different leucocytes and the varying degree in which the different forms take part in the production of the different types of hyperleucocytosis, it is evident at once that still less is to be anticipated from the centrifugal method in this direction than in the case of the red cells.

LITERATURE.—Hedin, Arch. f. ges. Phys., vol. xl. p. 360. Gärtner, Wien. klin. Woch., 1892, No. 2. Daland, Fort. d. Med., 1891, No. 21. Aspelin, Zeit. f. klin. Med., 1903, vol. xlix. p. 393.

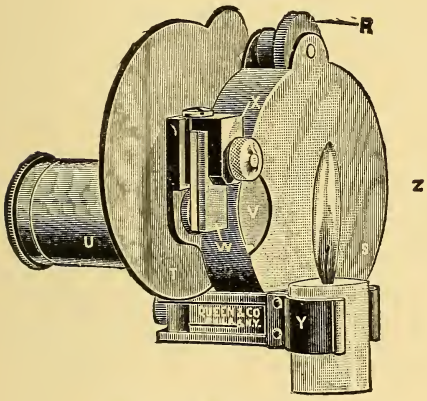
Estimation of Hæmoglobin.

Hæmoglobinometers.—While it is usually possible to form a fairly clear idea of the degree of anæmia by direct inspection of the patient, the appearance of the mucous surfaces, etc., it is often desirable to obtain more definite information, and, above all, a numerical expression of the extent of the anæmia. This is especially important in the diagnosis of certain forms of anæmia, in which the "color index" plays an important part—*i. e.*, the ratio between the percentage of hæmoglobin and the percentage of the red corpuscles as compared with the normal. To this end, special instruments have been devised, which are termed *hæmoglobinometers* or *hæmometers.* Of the various forms which are now in the market, the hæmoglobinometer of Dare is probably the best, and is rapidly replacing the old instrument of v. Fleischl, which for many years was the standard. It is more exact and more convenient. Miescher's modification of the Fleischl instrument is possibly still more accurate, but too costly for general adoption. The little instrument of Gowers, which has long been in the market, when obtained from a reliable source will also furnish good results, and is warmly commended by Sahli, Emerson, and others. Unfortunately many of those which

have been placed on sale are worthless. Oliver's instrument has some advantages over the Fleischl, but none over the Dare. The Talquist method is warmly commended by Cabot, and may be used to advantage in routine work by the general practitioner; for exact work it is insufficient.

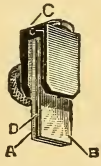
Dare's Hæmoglobinometer.—The essential parts of Dare's hæmoglobinometer (Fig. 23) are an automatic pipette for collecting the blood (Fig. 24) and a graduated color scale (Fig. 25) to measure the corresponding percentage of hæmoglobin. This latter reads from 10 to 120, the 100 mark corresponding to the color of a solution of 13.77 grammes of hæmoglobin in 100 c.c. of serum. The various shades of color corresponding to the scale are obtained by rotation of a prismatic glass semicircle tinted with golden purple of Cassius (Fig. 25, E), which is secured to a thin white glass disk (I). The numerical scale is placed on the edge of a corresponding semicircle (H) of thick white glass (F). This part of the apparatus is enclosed in a dust-proof hard-rubber case, and is rotated from the outside by the aid of a rubber-covered roller which runs on the edge of the disk and is turned by a milled wheel at R (Fig. 23). In the rubber case is a little circular window through which the color of the prism

Fig. 23.



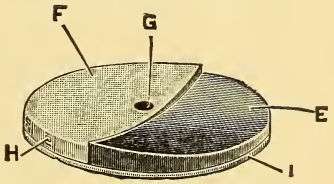
Dare's hæmoglobinometer.

Fig. 24.



Automatic pipette.

Fig. 25.

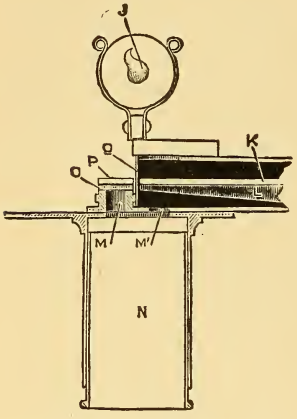


Graduated color scale.

is viewed by means of a small telescoping camera tube (Fig. 26, N), provided with a magnifying lens of low power. The color aperture represents a surface about equal to 3 per cent. of the color scale. Looking through the tube a corresponding window will be seen side by side with the one through which the color scale is visible. In front of this the blood pipette is secured. The essential part of this is an

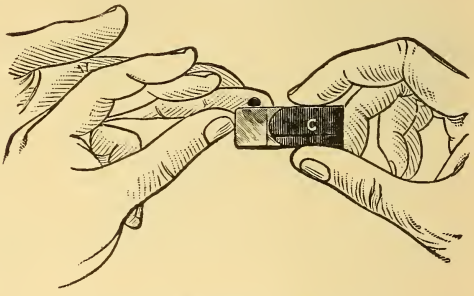
oblong plate of white glass (Fig. 24, A), into the end of which a depressed surface of measured depth is ground, the floor being exactly parallel to the plane surface of the glass. This depression forms a capillary chamber (D) when the transparent glass plate (B) is firmly clamped upon it by the pipette clamp C; it is filled by capillary attraction when either of the three free edges is touched to the blood drop. The pipette is held in position on the stage of the instrument by guides which run in grooves on the lower part of the clamp. The plate of white glass is toward the light.

Fig. 26.



Horizontal section of Dare's hæmoglobinometer (on a level with centre of comparison apertures): *J*, candle; *K*, white glass disk of color prism; *L*, color prism; *M*, aperture through which color of blood film is viewed; *M'*, aperture through which the illuminated color prism is viewed; *N*, camera tube; *O*, transparent glass of pipette; *P*, white glass of pipette.

Fig. 27.



Filling the automatic blood pipette.

The camera tube screws into a movable shutter (Fig. 23); when this is swung outward, the two apertures become visible through which the blood and the colored scale are viewed.

In front of the pipette a candle is clamped in such a position that both the blood and the color scale are equally illuminated.

Method of Use.—As the comparison of the color of the blood with that of the color scale should be made as soon after filling the pipette as possible, the apparatus is prepared for use beforehand by screwing the camera tube into place and adjusting the candle; this should be at such a level that the blue flame of the candle is below the color aperture, care being taken to have the wick of proper length (half inch) and not charred at the tip. Curved or eccentric wicks should be turned so that the intensity of light in a vertical position is midway between the two color apertures.

The glass plates of the pipette having been thoroughly polished and refastened in the clamp, the finger or ear is freely punctured as usual and the capillary space of the pipette filled with the blood, by holding one of the three edges horizontally to the drop (Fig. 27). Any blood adhering to the flat surfaces of the glass plates is carefully

wiped away and the pipette placed in position. The candle is lighted, the shutter thrown out, the camera tube focussed, and the color of the blood (on the left) compared with the color scale (on the right). The two are matched by rotating the color disk by means of the milled wheel, which should be done in an abrupt manner, and frequently resting the eye. To this end, the shutter is dropped and thrown out again as the case may be. The examination need not be conducted in a darkened room, but it is important to turn the instrument toward a dark background, so as to eliminate all direct or reflected light. The reading is indicated by the bevelled edge of the rectangular opening on the side of the case; the figure immediately beneath this represents the percentage of hæmoglobin. Immediately after use, the two glass plates of the pipette are cleansed with water and a little acid alcohol, dried, and again replaced. Further details in regard to technique accompany the instrument.

The amount of hæmoglobin in grammes corresponding to the readings is calculated according to the equation, $100 : 13.77 :: p : x$, and $x = 0.137\ p$, where p represents the reading actually noted and x the corresponding amount of hæmoglobin in 100 grammes of blood.

My personal experience with the instrument has been quite satisfactory. The readings are somewhat higher than those obtained with the Fleischl instrument and represent the actual condition more exactly.

LITERATURE.—A. Dare, Phila. Med. Jour., Sept. 22, 1900.

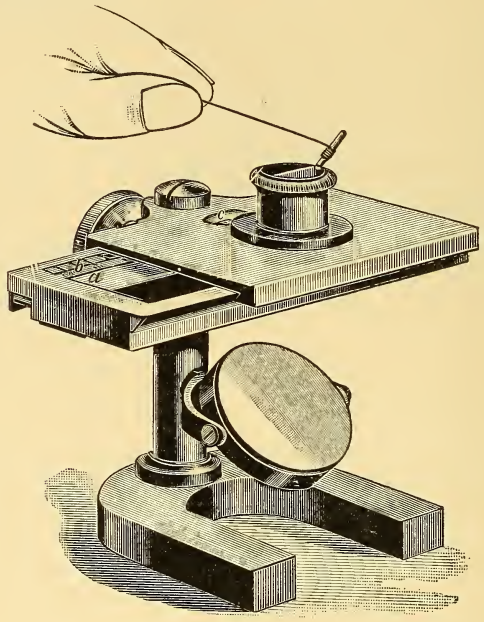
Fleischl's Hæmoglobinometer.—The principle underlying the v. Fleischl method is essentially the same as that of the Dare method; the color of the blood is compared with the color of a glass wedge stained with the golden purple of Cassius or a similar pigment, a scale indicating the corresponding amount of hæmoglobin. With the Fleischl method, however, diluted blood is used, which is one of the disadvantages of the method.

The instrument (Fig. 28) consists of the glass wedge *a*, to which a scale, *b*, is attached, ranging from 0 to 120, 0 being placed at the thinnest, 120 at the thickest portion of the wedge. By means of a rack and pinion this may be made to slide from side to side beneath a platform corresponding to the stage of a microscope. In the centre of the platform there is a circular opening into which artificial light (daylight is not permissible) is projected from a circular plate of plaster-of-Paris mounted beneath, in the position of the mirror of the microscope. Into the circular opening a metallic tube, 1.5 cm. in height, is fixed, which is closed at the bottom with a plate of glass and divided into two equal compartments by a metal partition. One compartment receives the light through the glass

wedge—the red chamber; the other, directly from the plaster-of-Paris reflector—the white chamber.

Capillary pipettes of known capacity accompany the instrument. This capacity is somewhat variable and is indicated on the handle of each, which number must correspond with that marked on the top screw-head of the instrument. Generally speaking, the capacity of each pipette is such that with the blood of a perfectly normal individual the mixture of blood and water in the white chamber will correspond in color to that of the colored wedge at the mark 100 (a 13.77 per cent. solution of hæmoglobin).

Fig. 28.



v. Fleischl's hæmometer.

The pipette is filled by capillary attraction from a drop of blood obtained in the usual manner. If on trial it is found that the blood does not immediately run up in the tube, this is repeatedly washed out with water and then dried. If this is always done *after* the examination, the pipette will be in working order on the next occasion. While filling the pipette care should be had that it is not immersed in the blood, but only brought in contact with it. The two compartments of the cell having been previously partly filled with water, the charged pipette is at once placed in the white chamber and rapidly moved to and fro until the blood is well mixed with the water. Any trace remaining in the pipette is carefully washed

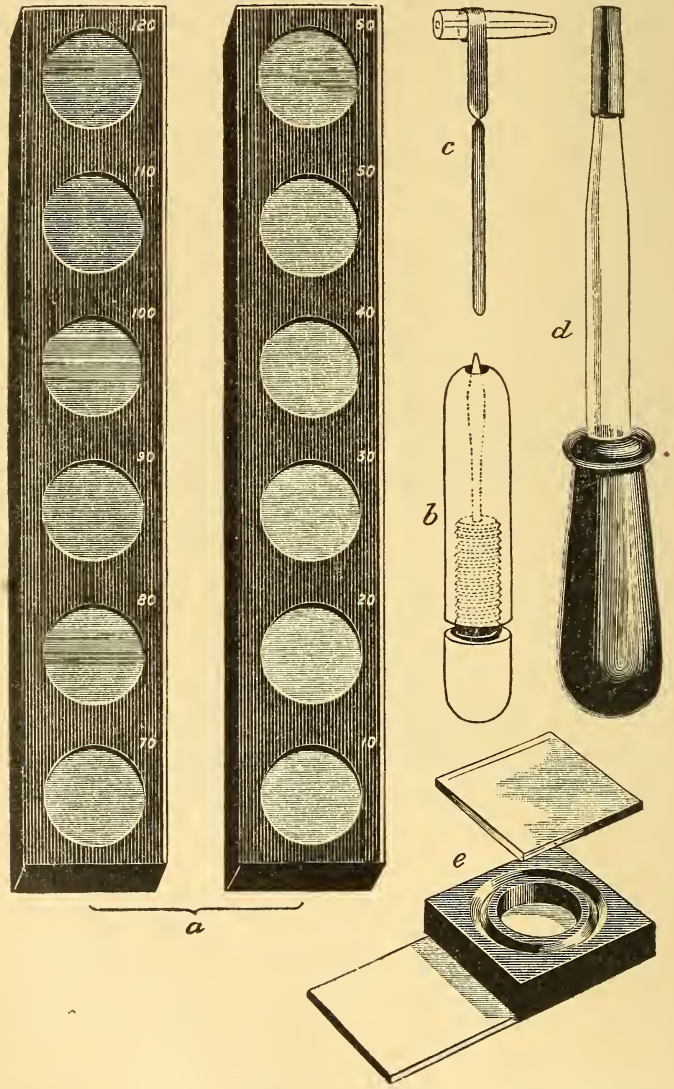
out with water by the aid of a medicine-dropper. The contents of the chamber are stirred with the handle of the pipette when both compartments are filled with water, using the same dropper, so that there is a convex meniscus over each. The color of the blood is then matched on the wedge, which should be moved by quick turns of the adjustment-screw rather than in a gradual way, as the eye will otherwise be less apt to appreciate fine shades of difference. Daylight, as I have said, is not permissible; a candle- or gas-flame of moderate intensity placed about a foot and a half distant is best. The eye should be perpendicularly above the cell, and it is well to view the colors through a paper tube which is placed over the two compartments. The number facing the notch in the little well immediately behind the cell indicates the percentage of hæmoglobin. The readings corresponding to the middle portion of the wedge are apt to be more nearly correct than the lower values. For this reason it is well, when a preliminary examination has shown a low figure, to repeat the test, using two or three pipettefuls of blood instead of one, the result, of course, being divided by 2 or 3, as the case may be. On the whole, the Fleischl method furnishes results which are somewhat lower than those obtained with the Dare; this is true especially of the older models, with which a percentage of 100 was only rarely observed. The instruments of more recent construction, however, are much better. Personally I regret to see the Fleischl apparatus supplanted by newer instruments; it was convenient and neat. It has its defects, to be sure, and it is unfortunate that the *Miescher modification*, in which these have been eliminated, and which unquestionably gives the most accurate results, is still so costly that its general use is out of the question.

Oliver's Hæmoglobinometer.—With this method the color of the blood in definite dilution is compared with a series of tinted glass standards. The principal advantage of the method lies in the fact that the color of the blood is compared at one time with one single tint. Two sets of standards are furnished; one intended for daylight, the other for candle-light, which latter should be chosen by preference as it yields more accurate results. Each set consists of twelve shades of color, the glass disks being mounted in two sets on a plaster-of-Paris background. They represent the color of a solution of hæmoglobin with a percentage running by tens from 10 to 120 (Fig. 29). Values intermediate between any two tens are obtained by superimposing riders of colored glass upon the disks, which represent 2.5 and 5 per cent. of hæmoglobin in the upper half of the scale, but twice this amount in the lower half.

A diluting chamber for diluting the blood, a pipette of 5 cbmm. capacity, a dropper, and a lancet accompany the color scale. The diluting chamber has a white background of plaster-of-Paris and a cover of blue glass. The procedure is the following: The finger is

punctured as usual and the pipette filled by capillary attraction, taking care that it only touches and is not immersed in the drop. The dropper, filled with water, is immediately attached to the narrow

Fig. 29.



Oliver's hæmoglobinometer: *a*, set of standard colored disks; *b*, lancet; *c*, capillary pipette; *d*, dropper; *e*, mixing chamber. (Ewing.)

end of the pipette, and the blood mixed with water forced drop by drop into the diluting chamber; care must be had that the blood is

not drawn into the pipette by adjusting the rubber end-piece while holding the pipette by its glass tube. The chamber is filled with water, the solution stirred with the handle of the pipette, and the blue-glass cover adjusted. When properly prepared, a tiny bubble of air should be included, showing that the chamber has not been overfilled. The candle is now placed at a convenient distance and the color of the diluted blood compared with that of the standard disks, looking through a collapsible camera tube. If it matches the color of any one of the disks, the percentage is read off at once. If, however, it is lighter than 40, for example, but darker than 30, one of the riders is placed upon 30, and a corresponding plate of unstained glass upon the diluting chamber, to compensate for the thickness of the rider. If the color cannot be matched in this manner, the average between the paler shade and the darker shade above is taken, remembering that on the lower half of the scale the actual value of the rider is double its face value. The room need only be darkened in part.

The method is less convenient than that of Dare or Fleischl, but yields good results; variations due to unavoidable error do not amount to more than 2 per cent. The cost of the instrument, however, is too great for its general adoption.

Gowers' Hæmoglobinometer.—The apparatus (Fig. 30) consists of two glass tubes (A and B) which are of the same diameter. One of these (A) is closed and contains a solution of picrocarmine-glycerin, the color of which corresponds exactly to that of a 1 per cent. solution of normal blood. The other tube is provided with an ascending scale of 120 divisions, each degree corresponding to 20 cbmm. A capillary pipette marked at 20 cbmm., a guarded lancet, a dropping-bottle, and a small stand accompany the instrument. On the stand the tubes can be so adjusted that it is possible to view them at an angle where the adjoining sides appear to overlap.

The finger is punctured as usual and the pipette filled to the 20 cbmm. mark; the blood is immediately discharged into the graduated tube and mixed with a few drops of water that has been previously placed there. The pipette is carefully washed out with water and the washings added to the blood; this is then further diluted with water drop by drop until the color of the blood, when held to the light or against a sheet of white paper, exactly matches that of the standard solution. After the addition of each drop the tube is inverted several times, closing the open end with the thumb; any adhering drops are wiped off against the edge of the tube so that they will flow back. The division on the scale ultimately reached indicates the percentage of hæmoglobin.

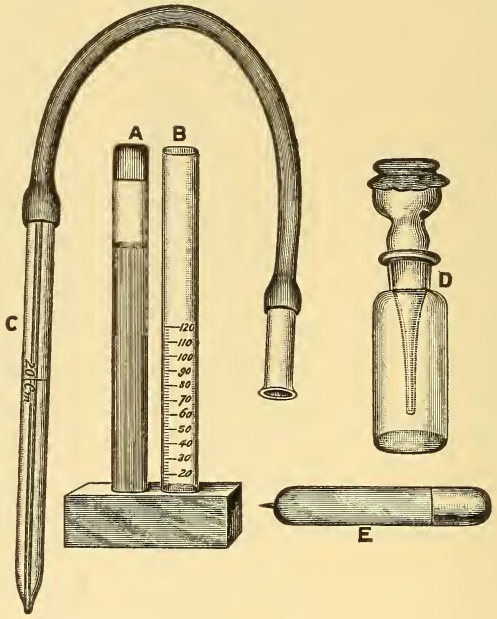
With very low percentages it is advised to use double the amount of blood, when the final result is, of course, divided correspondingly.

The method, as I have indicated above, is satisfactory if the

instrument has been obtained from a reliable source. Its low cost makes it especially serviceable in large clinics and for purposes of teaching in the clinical laboratory.

Talquist's Method.—The principle of the method is essentially the same as that underlying the Oliver method. The color of the blood, in this case undiluted, is compared with a series of lithographed standard tints, which represent a scale ranging by tens from 10 to 100. The technique is very simple: drops of blood are received on pieces of white filter-paper of suitable thickness which accompany the color scale, and are compared with the tints on the plate, using ordinary daylight.

Fig. 30.



Gower's hæmoglobinometer.

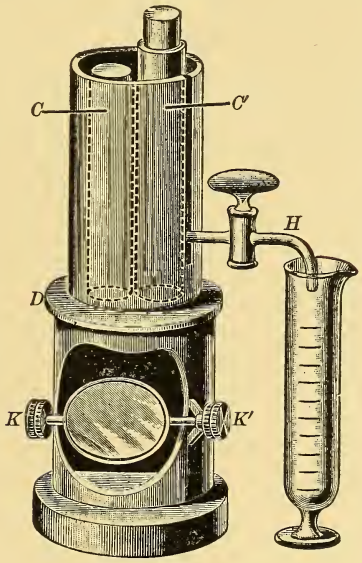
Accuracy is, of course, not to be expected from so crude a method, so that its use is of necessity limited. It will suffice in a very general way to control the result of treatment, but it is inapplicable in the determination of the color index.

Estimation of Blood-iron with Jolles' Ferrometer.—The estimation of the hæmoglobin from the amount of blood-iron, as originally suggested by Jolles, is unfortunately not possible, as it has been shown that constant relations between the two bodies do not exist. All the iron of the blood is not present in this form, nor does it all occur in the form of colored compounds. Jolles' method of estimating

the total amount of blood-iron deserves consideration, however, as it is a practical method and discloses facts which are of clinical interest. It is desirable that it should be introduced in the clinical laboratory as a routine method.

The principle is the following: a small amount of blood is incinerated, and the remaining red oxide of iron brought into

Fig. 31.



Jolles' ferrometer.

solution with a little monacid potassium sulphate. In this solution the iron is then estimated colorimetrically by means of a special apparatus—the ferrometer. As will be seen from the accompanying illustration (Fig. 31), this consists of two glass tubes, *C* and *C′*, which are of the same diameter throughout, and closed at the bottom with small glass plates, held in position by means of screws, as in the polarimetric tubes. Tube *C* is of 15 c.c. capacity, while tube *C′* is a little longer and holds about 16 c.c. Both are graduated in 0.5 c.c. Tube *C′* is provided with an overflow tube near the bottom, which carries a stopcock. Both are fitted into the perforated metallic plate *D*, and are surrounded by a casing, so as to exclude light from the sides. Below the plate is a plaster-of-Paris reflector, which can be turned with the screws *K* and *K′*. Tube *C* receives the iron solution, obtained from the blood, and is closed with an accurately fitting glass disk, while in *C′* is placed the iron solution used for comparison. This is allowed

to flow away through the overflow tube (*H*) drop by drop until the color in the two tubes is the same. But as the color in *C'*, owing to the meniscus which is formed, would be less sharply defined than in *C*, the tube *C'* is furnished with a cylindrical float of aluminum, which is closed above and below with glass disks. This float dislodges about 1 c.c. of fluid, and it is for this reason that tube *C'* is a little longer than tube *C*.

A capillary pipette and the necessary additional apparatus, as well as reagents, accompany the instrument, which is made by Reichert, of Vienna.

Method.—In order to procure the necessary amount of blood, viz., 0.05 c.c., which is obtained by simple puncture of a finger or the ear, Jolles recommends that the capillary tube be first filled *beyond* the mark, and to close the pinchcock on the rubber tube at once. The excess of blood is then allowed to flow from the tube, and the tip is carefully wiped with filter-paper. The 0.05 c.c. is placed in a platinum crucible, any traces that may remain adherent to the tube being washed out with a little distilled water.[1]

The blood is evaporated to dryness over a plate of asbestos, at first with a small flame. The crucible is placed on a pipe-stem triangle, and the residue carefully incinerated. One of the accompanying powders, containing 0.1 gramme of monacid potassium sulphate is now added. The mixture is cautiously heated with a small flame until the powder begins to liquefy, when stronger heat is applied and the mass congeals. This step is completed in one or two minutes. On cooling, the material is washed into the cylinder *C*, through a small funnel with the aid of a little hot distilled water, and diluted to the mark 10. The tube *C'* is charged with 1 c.c. of the comparison-solution, and likewise filled to the mark 10 with hot distilled water. This solution contains 0.0005 gramme of iron and 0.1 gramme of monacid potassium sulphate, in every cubic centimeter.

To each cylinder are then added 1 c.c. of hydrochloric acid (1 : 3) and 4 c.c. of a solution of ammonium sulphocyanide (7.5 grammes pro liter). The tube *C* is now closed with the glass disk, care being taken to exclude bubbles of air, when the mixture is thoroughly shaken and the tube fixed in the metallic plate. Tube *C'* is likewise closed with a glass disk; its contents are well agitated, the disk is removed and replaced by the carefully dried float. This should be placed upon the fluid slowly and with a screwing motion, so as to exclude bubbles of air. After this tube has also been placed in position the reflector is adjusted, and so much of the comparison-solution allowed to escape as to make the color in the two tubes the

[1] The pipette should always be cleansed immediately after use. It is best washed out with dilute sulphuric acid (10 per cent.), then with dilute sodium hydrate solution (5 per cent.), and finally with alcohol and ether.

same. C' is then removed from its base and the reading taken. In the table below, the corresponding amount of iron in 1000 c.c. of blood may be directly read off. Should it be desired to obtain the percentage by weight, the specific gravity of the blood should first be ascertained, and the necessary calculation made according to the equation $D : V :: 100 : x$, and $x = \frac{100.V}{D}$, in which D represents the specific gravity, and V the percentage by volume. The resulting differences, however, are so small that they may be neglected, and for practical purposes it will be sufficient to assume a specific gravity of 1.050, and to read off the percentage by weight directly. To this end, the second column in the table has been constructed.

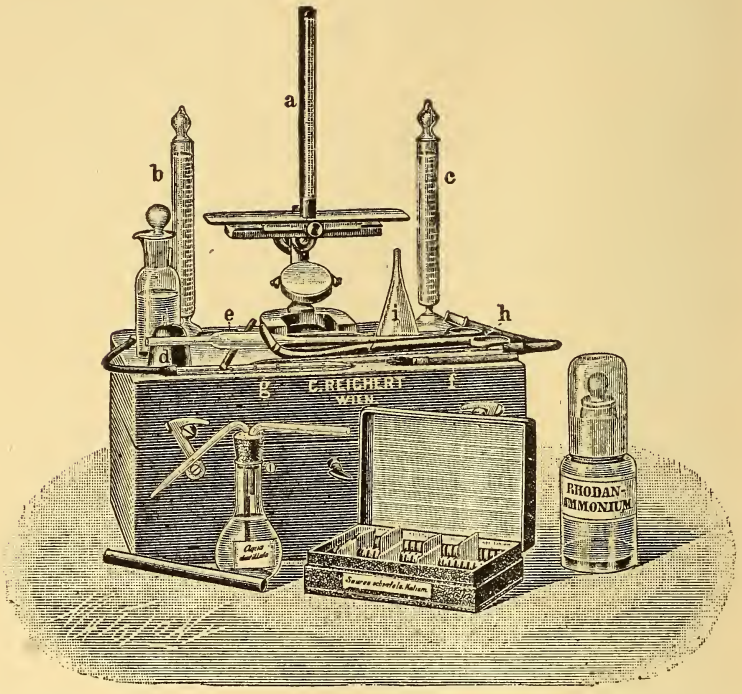
TABLE TO ASCERTAIN THE AMOUNT OF IRON IN 1000 C.C. OF BLOOD, AND THE PERCENTAGE BY WEIGHT, FROM THE NUMBER OF C.C. OF THE COMPARISON-SOLUTION USED.

C.c. of comparison-solution used.	Iron in 1000 c.c. of blood.	Iron-percentage by weight.
15.0	1.000	0.0952
14.5	0.967	0.0920
14.0	0.933	0.0889
13.5	0.900	0.0857
13.0	0.867	0.0825
12.5	0.833	0.0794
12.0	0.800	0.0762
11.5	0.767	0.0730
11.0	0.733	0.0698
10.5	0.700	0.0666
10.0	0.667	0.0635
9.5	0.633	0.0603
9.0	0.600	0.0571
8.5	0.567	0.0540
8.0	0.533	0.0508
7.5	0.500	0.0475
7.0	0.467	0.0444
6.5	0.433	0.0412
6.0	0.400	0.0381
5.5	0.366	0.0349
5.0	0.333	0.0317
4.5	0.300	0.0285
4.0	0.266	0.0254
3.5	0.233	0.0222
3.0	0.200	0.0191
2.5	0.166	0.0158
2.0	0.133	0.0127
1.5	0.100	0.0095
1.0	0.067	0.0063

More recently Jolles has modified his ferrometer in such a manner that the comparison of the sulphocyanide solution, obtained from the blood, is made with the colored wedge of Fleischl's hæmometer. The new instrument (Fig. 32) he terms the *clinical ferrometer*, and, as made by Reichert in Vienna, it can readily be transformed into the hæmometer proper. Full directions accompany the apparatus. The results are expressed in relative terms, the number 100 on the scale

corresponding to 0.0425 per cent. by weight of iron. Some of the results which have been obtained with the clinical ferrometer are

FIG. 32.



given below, together with the corresponding figures indicating the amount of hæmoglobin.

	Ferrometer number.	Hæmometer number.
Normal	103.0	100
Normal	92.6	105
Normal	95.5	100
Normal	110.0	105
Normal	83.8	92
Chlorosis	32.1–68.2	30–65
Simple anæmia	33.2–74.7	15–40
Icterus	55.0	80
Leukæmia	40.7	32
Leukæmia	38.6	35
Pseudoleukæmia	77.24	75–80
Severe diabetes	78.7	30
Severe diabetes	91.4	35–40
Parenchymatous nephritis	51.7	50

These figures at once illustrate the lack of relationship which exists between the amount of hæmoglobin and that of the blood-iron as a whole.

Jellineck has made a careful comparative study of the blood with Jolles' instrument and v. Fleischl's hæmometer and arrived at some very interesting conclusions. In diabetes he thus found that the amount of iron steadily diminishes, although the hæmoglobinometer gives higher readings. In a case of malaria the iron remained constant before and after the chill, while with v. Fleischl's instrument variable results were obtained. In two cases of leucocytosis the ferrometer gave low readings, and in eight cases of secondary anæmia the hæmometer gave much higher values than the ferrometer.

In a series of cases Jolles also examined into the presence of iron in the serum, by centrifugating a given volume of blood mixed with an 0.8 per cent. salt solution, and found that in health the serum contains no iron. In three cases of chlorosis, in one case of leukæmia, in one of neoplasm, and one of interstitial nephritis, negative results were likewise reached. In two cases of severe diabetes, on the other hand, notable quantities were found.

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Kryoscopic Examination of the Blood.

The kryoscopic examination of the blood has for its object the determination of the molecular concentration, and hence of the osmotic pressure of the blood. The method is essentially based upon the observations of Raoult: (*a*) that all solid, liquid, or gaseous substances when dissolved in a liquid will lower the freezing-point of that liquid; (*b*) that the degree to which the freezing-point is lowered is dependent upon the amount of substance which is present in solution; and (*c*) that equimolecular solutions have like freezing-points.[1]

It follows that the freezing-point of a solution furnishes an index of its molecular concentration, and hence also of its osmotic pressure, as this has been shown by van't Hoff to be proportionate to the number of molecules present.

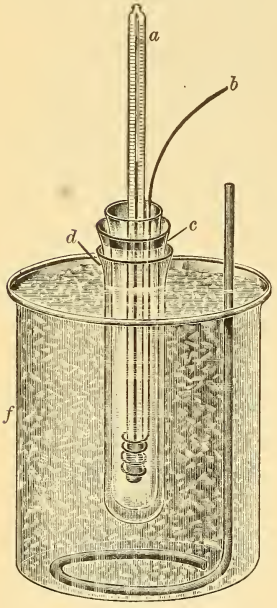
The degree to which the freezing-point is lowered is designated by the letter $\triangle$. In the case of normal blood this varies between —0.56 and —0.58° C., as compared with distilled water. A further depression is probably always indicative of renal insufficiency; it is a symptom of decided value and deserves more general considera-

[1] Solutions are termed equimolecular when for a constant quantity of the solvent they contain such quantities of substance in solution that these bear the same ratio to each other as their molecular weights. Example: The molecular weight of sodium chloride is 58.5 and of sodium carbonate 106; if we dissolve these quantities or the same multiples of each in a constant quantity of water, such solutions would be equimolecular.

tion. In the domain of renal surgery especially the study of kryoscopy of the blood is especially important. Of foreign investigators, Kümmel more particularly has pointed out the value of the method in this field. As the result of 265 freezing-point determinations of the blood, in 170 cases in which various operations were performed upon the kidney and in which a direct examination of the organ was possible, he concludes that kryoscopy furnishes the most important index of renal insufficiency as compared with all other modern methods. Other observers, such as Casper and Richter, Tinker, and others, have arrived at similar conclusions. To Koranyi, however, belongs the credit for the introduction of kryoscopy into the clinical laboratory and its application to the study of renal diseases. Senator, Claude and Balthazar, Albarran, Kövesi, Lindemann, Waldvogel, and others have materially contributed to establish its value as a clinical method.

Method.—In the clinical laboratory a modification of Beckmann's apparatus is most conveniently employed (Fig. 33). Its essential parts are: (*a*) a Heidenhain thermometer graduated in hundredths and reading from —1° to —5° C.; (*b*) a platinum wire loop for stirring; (*c*) a test-tube which is closed by a stopper through which the thermometer and stirring-wire pass, and which in turn is placed in a second larger tube (*d*) so as to be surrounded by an air-space. The jar *f* is filled with a freezing-mixture of salt and ice, the temperature of which should lie between —2° and —5° C. Into this is placed the second tube *d*. The test-tube *c* is charged with 20 c.c. of blood (if only 10 c.c. are available, this amount may suffice), obtained by means of a large aspirating-syringe from one of the veins near the bend of the elbow, as in the case of a bacteriological examination of the blood (page 166); the thermometer is introduced and the stirring-wire adjusted. The tube is placed directly in the freezing-mixture until the mercury leaves the reservoir bulb; this is done to save time. It is then adjusted in the second tube *d*, as shown in the illustration, and the blood constantly stirred with the platinum wire. The temperature falls more or less rapidly below the freezing-point before actual freezing takes place; as this occurs it suddenly rises again owing to liberation of heat, and then remains constant for some time. This

Fig. 33.



Beckmann's apparatus.

point represents the true freezing-point. Later, if the tube is allowed to remain in the freezing-mixture, the temperature may fall to that of the latter. The difference between the freezing-point of distilled water and that of the blood is $\triangle$.

In every case it is necessary to determine the true zero for each instrument separately, as this often varies somewhat owing to unavoidable errors incident to its construction. To this end, the tube *c* is charged with three to four times the amount of distilled water which is necessary for one examination. The greater portion of this is frozen; the liquid portion is thrown away; the frozen water is allowed to thaw and is again frozen in part, a portion being again thrown away; the remainder is sufficiently pure for the examination.

The freezing-mixture is prepared by packing alternate layers of ice and salt into the jar *f* around the tube *d*, which is held in position while the ice is packed. Ice and salt are finally thoroughly mixed by stirring with a heavy wire ring and rod (*g*). If several examinations are to be made, the water which separates out is poured off and replaced by an additional amount of salt and ice.

The method is quite expeditious, and if everything is previously prepared, the examination does not occupy more than ten or fifteen minutes.

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BACTERIOLOGY AND PARASITOLOGY OF THE BLOOD.

Typhoid Fever.

Recent researches have shown that in typhoid fever the specific organism (Plate XV., Fig. 3) can be isolated from the blood directly in a fairly large percentage of cases and at a time when the Widal reaction (see below) may not as yet be obtainable, viz., on the fifth day of the disease. Schottmüller thus found the organism in 40 of 50 cases, Castellani in 12 of 14, Kühnau in 11 of 41, Courmont in all of 9 cases, Auerbach and Unger in 7 of 10, and Cole in 11 of 15 cases. Neuhaus, Neufeld, Curschmann, Rumpf, and others had previously shown that the bacillus may at times be cultivated from the blood taken from the roseolar spots. More recently Polacco and Gemelli report that with a modification of Neufeld's method they obtained the bacillus from the rose spots in every one of 50 typhoid patients.

The blood is withdrawn by means of a sterilized syringe from one of the superficial veins of the arm; 300 to 500 c.c. of bouillon are inoculated with from 2 to 4 c.c. of the fresh blood and examined after from eighteen to twenty-four hours. If a negative result is obtained in the hanging drop, a further examination is made twenty-four hours later. At first the bacilli are but little active, but on further cultivation and reinoculation their motility increases. For purposes of identification they are grown on agar slant, in milk, bouillon, glucose, and further tested with an actively agglutinating serum (see below). It is interesting to note, however, that their tendency to agglutination is almost invariably much inferior to that of bacilli which have been maintained for a long time on artificial media. Courmont thus notes that they were commonly agglutinated with a dilution of 1 : 50 by a serum which agglutinated laboratory bacilli at 1 : 200.

Cole uses from 8 to 10 c.c. of blood, which is immediately diluted with bouillon contained in Erlenmeyer flasks, about 150 c.c. of bouillon being used for each flask. From one to six flasks are prepared, the dilution being 1 : 75 to 1 : 150. The flasks are then well skaken and placed in an incubator for twenty-four hours, after which, if the bouillon is cloudy, agar plates are made. With this technique a positive result can be obtained in thirty-six hours.

Literature.—Neuhaus, Berlin. klin. Woch., 1886, Nos. 6 and 24. Schottmüller, Deutsch. med. Woch., 1900, No. 32. Castellani, cited in Presse méd., June, 1900. Auerbach u. Unger, Deutsch. med. Woch., 1900, No. 29. Cole, Johns Hopkins Hosp. Bull., 1901, p. 203. Courmont, Jour. d. physiol. et d. pathol. gén., 1902, vol. iv. p. 155. Polacco and Gemelli, Centralbl. f. inn. Med., 1902, vol. xxiii. p. 121.

Widal's Serum Test.—Of greater practical utility than the cultivation of the typhoid bacillus from the blood is the fact that the blood-serum of patients affected with typhoid fever possesses the property of causing arrest of motility and agglutination of the specific bacilli. This observation, originally made by Pfeiffer, was first utilized for diagnostic purposes by Widal, in 1896. The method which bears his name has now been quite generally adopted in the clinical laboratory, and must be regarded as a most valuable aid in the diagnosis of typhoid fever. The reaction occurs in over 95 per cent. of undoubted cases, and may appear as early as the first day of the disease, meaning thereby the first day that the patient spends in bed or the fifth day of general malaise. Such instances, however, are very uncommon, and, as a general rule, a positive result is obtained only after the fifth or sixth day in bed. In a small number of positive cases, on the other hand, the patient may pass through the entire course of the disease, and present typical clumping only during convalescence or a subsequent relapse. In every case, therefore, in which no reaction is obtained upon first trial, the test should be

repeated at regular intervals throughout the disease until a definite result is obtained. Intermittence of the reaction, moreover, is very common, and emphasizes still further the necessity of frequent examinations in apparently negative cases.

While in some instances the reaction disappears very soon after the temperature reaches normal, and even earlier, it generally continues into convalescence, and may be observed for months and years after the attack. Cases have thus been recorded in which a positive reaction could be obtained as long as thirty-seven years after infection.

The question, whether or not Widal's reaction is a specific reaction of the typhoid organism, can, I think, be answered in the affirmative, notwithstanding the facts that at times cases of true typhoid fever are seen in which no clumping is obtained, and that the reaction has been observed in cases which were apparently non-typhoid. Such exceptions, no doubt, are due in part to faulty technique, viz., to too low a degree of dilution of the serum, the use of old or impure cultures, too long a time-limit of observation, single negative tests, etc. On the other hand, there can be no doubt that typhoid bacilli are at times present in the body without giving rise to symptoms of typhoid fever. In a case of cholelithiasis, reported by Cushing, typhoid bacilli were thus found in the gall-bladder, and distinct clumping was observed with a dilution of 1 : 30, although no history of typhoid fever could be obtained. There can further be no doubt that individuals exist who are naturally immune against typhoid fever, and that some of the positive results which have been obtained in perfectly healthy individuals who have never had typhoid fever may be explained in this manner.

While the reaction may hence be regarded as a specific infectious reaction of the typhoid organism, nevertheless its value in diagnosis is limited. This is owing largely to the fact that in many cases a positive result is not obtained before the end of the second or third week, and may even be delayed until a relapse occurs. Its persistence for years after infection is also an obstacle to its general utility, not to speak of its occurrence in apparently healthy individuals and in diseases in which an association with the typhoid organism is not apparent. A rather interesting apparent exception to the rule that the Widal reaction is only obtained in cases of typhoid infection is reported by Grünbaum,[1] who notes that he obtained a positive reaction in cases of febrile jaundice; he suggests that in these cases the infection was caused by one of the intermediate organisms between the typhoid bacillus and the bacillus coli communis. In so-called paratyphoid fever, however, a positive Widal reaction is either absent or imperfect and only observed with a low dilution (see page 170).

[1] Grünbaum, cited by Durham, Brit. Med. Jour., 1898, vol. ii. p. 600.

Widal's test is a most valuable aid in the diagnosis of typhoid fever, but cannot be relied upon to the exclusion of other symptoms.

Technique.—The method is based upon the fact that typhoid serum will cause arrest of motility and agglutination of the specific bacilli even when diluted, whereas clumping of the same organism is obtained only with sera from other diseases and healthy individuals when these are used in a more concentrated form. The time-limit at which clumping occurs is likewise an important factor, as non-typhoid sera are at times met with in which, notwithstanding a certain degree of dilution, agglutination occurs, providing that the specimen is kept for a long time. Both factors, viz., the degree of dilution necessary to eliminate the agglutinating power of non-typhoid sera, as also the time-limit of observation, have been arbitrarily determined. Widal originally advised a dilution of 1 : 10, and Grüber a time-limit of one-half hour. At the present time there is a tendency, among German physicians especially, to increase the degree of dilution to 1 : 40, and even 1 : 50, and the time-limit to from one to two hours. Generally speaking, a positive reaction is of greater value the greater the degree of dilution at which it can still be obtained. A uniform standard, however, is necessary in order to allow a strict comparison of results, and I am personally inclined to favor the German standard. The degeee of dilution should exceed 1 : 40, as undoubted positive reactions in non-typhoid individuals have been obtained with 1 : 20 and even 1 : 40.

In any event, only a full-virulent, fresh bouillon culture of the typhoid bacillus, viz., one not older than sixteen to twenty-four hours, should be used. The further technique is simple: 1 volume of blood-serum is diluted with the requisite amount of the bouillon culture, viz., to 10, 20, 30, 40, or 50 volumes, as the standard may be. Of this mixture, one drop is mounted on a slide, covered, and examined with a moderately high power. If the case in question is one of typhoid fever, it will be observed that after a variable length of time the individual bacilli, which at first actively dart about the field of vision, become quiescent and tend to gather in distinct clumps, while the interspaces become entirely free from bacilli or very nearly so. After one-half hour, or one or two hours, according to the degree of dilution, all motion has ceased. When the time-limit has expired and loss of motility and agglutination have not occurred the result is negative. In such an event further examinations should be made on the following days. In every case it is well to make a control-test with the simple bouillon culture, so as to insure the absence of preformed clumps and the virulence of the organism; of the latter, the degree of motility is the best index.

In order to secure the necessary degree of dilution, various methods have been suggested. The simplest and the one generally employed in municipal bacteriological laboratories, is to receive a large

drop of blood upon a slide or slip of glazed paper, and allow it to dry. A drop of distilled water is then placed on the blood and allowed to remain for several minutes, when it is washed off and intimately mixed with the requisite number of drops of the bouillon culture, and examined as described. The principal advantages of this method are its simplicity and the fact that the *dried* blood retains its agglutinating properties for weeks and months. The results, however, are less reliable than with the use of liquid blood. If this is to be employed, properly graduated capillary pipettes are prepared, similar to the pipettes accompanying the Thoma-Zeiss hæmocytometer. Blood is first drawn up to a given mark and expelled into a small watch-crystal; the requisite amount of the bouillon culture is then obtained with the same pipette and immediately mixed with the blood, and a drop of the mixture is examined under the microscope. Sterilization of the apparatus used is unnecessary, and each pipette is destroyed after use.

If it is desired to keep the liquid blood for any length of time, similar pipettes may be used with a small bulb blown in the middle. These are first sterilized by heat and sealed at the ends. Before use, one end is broken off, the bulb heated in a spirit flame, and filled by capillary attraction. It is then again sealed, when the blood may be kept indefinitely. Another method, which is said to be even more reliable than those mentioned, is the following:

After careful disinfection of the arm, 5 or 6 c.c. of blood are withdrawn from one of the superficial veins, by means of a sterilized hypodermic syringe, and placed in a sterilized test-tube measuring from 10 to 12 cm. in length. The blood is allowed to stand until the serum has separated from the clot, which may be hastened by loosening the coagulum from the walls of the tube with a platinum needle. Eight drops of the serum are added to 4 c.c. of nutrient bouillon, which should be as nearly neutral as possible, when the mixture is inoculated with 1 oese (platinum loopful) of a fresh bouillon culture of the typhoid bacillus not more than twenty-four hours old. The tube is kept at a temperature of 37° C. for twenty-four hours. At the end of this time, and frequently earlier, the bouillon will be absolutely clear, or very nearly so, while little flakes, composed of the bacilli, will be seen at the bottom and adhering to the sides of the tube, if the case under observation is one of typhoid fever; otherwise the bouillon becomes uniformly cloudy and a true sediment is not formed. A pseudo-reaction also may occur at times, which should not be confounded with the one just described. Innumerable microscopical, dust-like particles will then be seen scattered throughout the fluid, which can readily be distinguished from the cloudy appearance of non-typhoid specimens. It has been suggested that this result is obtained in cases of intense infection with the Bacillus coli communis. Should doubt arise, it is only

necessary to keep such tubes for a few hours at a temperature of 37° C., when it will be noticed that the dust-like aspect has given place to the ordinary cloudy appearance observed in cases which are not typhoid fever.

Of the nature of the substance or substances which cause agglutination—*agglutinins*—little is known that is definite. It appears that in the blood they are intimately associated with fibrinogen and globulin, as plasma from which these two bodies have been removed no longer possesses agglutinating properties. As chemical differences, however, apparently do not exist between normal globulin and globulin obtained from typhoid blood, it seems likely that the substances in question do not form an integral part of the globulin molecule, but perhaps are thrown down mechanically when the proteid substances are precipitated. This view is rendered probable by the fact that typhoid urine free from albumin may likewise cause arrest of motility and agglutination of typhoid bacilli. Attempts to separate the agglutinins from the proteids of the blood have thus far not been successful.

The milk of immunized animals or of typhoid patients acts like the blood, and in it the agglutinins are apparently associated with caseïn. Exposure of such milk to a temperature of 80° C. destroys its agglutinating power. Very interesting is the observation of Malvoz, that very dilute solutions of safranin and vesuvin act upon the typhoid bacilli as typhoid serum does, and upon these bacilli only.

Literature.—Pfeiffer, Zeit. f. Hyg., vol. xviii. p. 1. Pfeiffer u. Kolb, Deutsch. med. Woch., 1896, p. 185. Grüber u. Durham, Münch. med. Woch., 1896, pp. 206 and 285. Widal, Soc. méd. des Hôp., 1896, p. 561; and Presse méd., 1897, i. p. c. Biggs and Park, Am. Jour. Med. Sci., vol. cxiii. p. 274. Stewart, Trans. Am. Pub. Health Assoc., vol. xxiii. p. 151. Förster, Zeit. f. Hyg., vol. xxiv. p. 500. Da Costa, N. Y. Med. Jour., 1897. Anders and McFarland, Phila. Med. Jour., 1899, pp. 778 and 832. Bieberstein (collective work), Zeit. f. Hyg., 1898, vol. xxvii. Tobiesen (350 cases), Zeit. f. klin. Med., 1901, vol. xliii. p. 147.

Paratyphoid Fever.

In cases of so-called paratyphoid fever organisms may be met with in the blood which apparently occupy a position intermediate between the typhoid bacillus and the organisms belonging to the colon group. Collectively they are spoken of as paratyphoid or paracolon bacilli, though the question whether or not they represent a well-defined species has not been definitely settled.

Cases of paratyphoid fever clinically resemble true typhoid, but their serum does not react with the typhoid bacillus, or at least only imperfectly so and with a low dilution, while the organism which appears to be pathogenic in the individual case is agglutinated in a typical manner. Unfortunately, however, the serum of one case will not always react with the organism of a second case, so that it seems rather doubtful if the serum reaction will prove of value in distinguishing the intermediates as an entire group from typhoid on the

one hand, and the bacillus coli on the other. Many varieties apparently exist (Gwyn's paracolon bacillus, Cushing's bacillus 0, Hewlett's bacillus *b*, Noonan's bacillus, etc.).

Organisms belonging to this group have been described by Widal, Gwyn, Cushing, Schottmüller, Kurth, Brill, Johnston, Coleman-Buxton, and others. It is likely that the small percentage of cases which are clinically typhoid fever, but in which the Widal reaction is persistently absent, are cases of this order.

The examination of the blood is conducted as in typhoid fever, although it is perhaps not always necessary to dilute the blood to the same degree. In Gwyn's case successful cultivation followed the spreading of a few c.c. of blood over the surface of agar tubes or plates. Noteworthy is the fact that the intermediates do not form gas in lactose media; in saccharose media also they produce no gas, though this fact is less important, as many true colon bacilli likewise are incapable of causing its fermentation. Milk is not coagulated. Schottmüller states that the organisms at first render the milk acid, but that subsequently the reaction becomes alkaline. On potato the growth is slight and there is no discoloration.

LITERATURE.—Gwyn, Johns Hopkins Hosp. Bull., 1898, vol. ix. p. 54. Cushing, Ibid., 1900, vol. xi. p. 156. Schottmüller, Zeit. f. Hyg., 1901, vol. xxxviii. W. B. Johnston, Am. Jour. Med. Sci., 1902, vol. cxxiii. p. 187 (analysis of all cases reported up to that time). A. W. Hewlett, Ibid., p. 200. Coleman-Buxton, Ibid., 1903, vol. cxxiii. p. 976. See also Ascoli, Zeit. f. klin. Med., 1903, vol. xlviii. p. 419.

Pneumonia.

Recent research has brought to light the interesting fact that in fatal cases of acute croupous pneumonia the specific diplococcus is quite frequently present in the blood, while in cases ending in recovery it is encountered only exceptionally. I have found, as a matter of fact, that a positive result is obtained in more than 50 per cent. of the fatal cases. The invasion of the blood usually occurs twenty-four to forty-eight hours before death, but may take place at an earlier date or be delayed. From the standpoint of prognosis a bacteriological examination of the blood may thus be of considerable importance. It should be remembered, however, that while a positive result is always a symptom *mali ominis*, there are cases on record in which recovery occurred notwithstanding the presence of diplococci in the blood. In such cases metastatic infection probably has occurred.

Prochaska, working under Eichhorst's direction, reports that he found pneumococci in the blood in each of 10 cases examined, and in a subsequent series of 40 cases, of which 7 were fatal, he obtained the pneumococcus in 38. Twice there developed organisms concerning the nature of which the author is uncertain. These were streptococci with a tendency to arrangement in pairs, and may have been an

especially virulent variety of pneumococcus. One of these cases ended fatally; in the second slow resolution was followed by a secondary indurative process. The cultures were made with 10 c.c. of blood, at varying periods of the disease, sometimes as early as the second day. It is noteworthy that in two cases positive results were found on the day following the crisis; in one, followed by empyema, on the second day after the crisis; and in another case, in which slow resolution was taking place, organisms were found three days after the febrile crisis.

Cole examined 30 cases at the Johns Hopkins Hospital and obtained positive results in 9. All of these ended fatally, but it is to be noted that 4 additional cases of the series died, and that in these the pneumococcus was not obtained.

Fränkel states that according to his experience, which is based upon an examination of more than 150 cases, one may infer that death will occur either with the symptoms of sepsis or that metastasis will take place in the internal organs whenever a larger number of colonies develop on spreading 1 c.c. of blood upon a plate of agar. If, however, the number is so small that it is necessary to take larger amounts of blood to demonstrate their presence, and to grow them in bouillon instead of on agar, so as to eliminate the bactericidal power of the blood altogether, then Fränkel believes their presence is of no significance, and does not warrant a fatal prognosis. In the latter case he has found that the bacteria are frequently avirulent.

Of 72 cases of pneumonia which came to autopsy at the Boston City Hospital in a total of 341 deaths from the disease, the pneumococcus was found in the blood in 36, either alone or together with other organisms. No ante-mortem cultures, however, were made. These negative results, in the face of the positive findings of Prochaska, are no doubt referable to some difference in technique.

The examination, which should be repeated every day, is conducted as follows: After disinfection of the arm one of the superficial veins is compressed with a finger and punctured with an ordinary hypodermic syringe which has previously been sterilized in boiling water. 10 c.c. of blood are aspirated and agar tubes—liquefies at 40° C.—inoculated, each with 1 or more c.c. of the blood. Plates are then prepared and kept at a temperature of from 35° to 37° C. The colonies number from 2 to 200, and appear as small round, grayish, jelly-like drops, which are quite characteristic. During their growth they cause a greenish discoloration of the blood-agar. Other bacteria possess the same property, but to a less marked degree than the diplococcus pneumoniæ.

Instead of agar, bouillon may also be employed, and it is quite likely, as Prochaska suggests, that in this manner positive results may be more frequently obtained. Cole recommends the use of

sterile litmus milk, of which portions of 150 c.c. each are employed in Erlenmeyer flasks. Early acidification and coagulation occur, and it is thus possible to determine more readily and quickly whether growth has taken place. Smears are then made and examined for capsules (see below). The identity is established by the characteristic shape and staining reactions of the organism, including the staining of the capsules, by the typical growth in milk and agar, and by the absence of growth, or very slight growth, in gelatin at ordinary room temperature.

The individual organism (Plate XVII., Fig. 2) is capsulated, and usually occurs in pairs, arranged end to end or in short chains. At times, however, the chains are quite long, and then it may be difficult to distinguish it from streptococci. It is easily stained with the common anilin dyes. In order to differentiate the capsule, the following method, suggested by Welch, is best employed: Spread and dried cover-glass preparations are treated first with glacial acetic acid, which is allowed to drain off, and is replaced (without washing in water) with anilin-gentian-violet solution. The staining solution is added repeatedly until all the acid is replaced. The specimen is now washed in a weak salt solution (about 2 per cent.) and examined in this, and not in balsam. The capsule and coccus can thus be differentiated.

LITERATURE.—Goldscheider, Deutsch. med. Woch., 1892, No. 14. Sittmann, Deutsch. Arch. f. klin. Med., 1894, vol. liii. p. 323. Kühnau, Zeit. f. Hyg., 1897, vol. xxv. Kohn, Deutsch. med. Woch., 1897, p. 136. James and Tuttle, N. Y. Presbyterian Hosp. Rep., vol. iii. p. 44. Sello, Zeit. f. klin. Med., 1898, vol. xxxvi. White, Jour. of Exper. Med., 1899, vol. ii. Silvestrini and Sertoli, Riforma Med., 1899, No. 116. Abstr. in Centralbl. f. inn. Med., 1899, vol. xxi. R. Cole, Johns Hopkins Hosp. Bull., 1902, vol. xiii. p. 236. Prochaska, Centralbl. f. gen. Med., 1900, No. 46. Prochaska, Deutsch. Arch. f. klin. Med., vol. lxx. p. 559. Fränkel, Deutsch. med. Woch., 1901, V. B., p. 212.

Sepsis.

The importance of a careful bacteriological examination of the blood in septic conditions has been definitely established. The technique is the same as that described above (page 169). But whether or not it is always necessary to use so much blood as in typhoid fever and pneumonia and so high a degree of dilution, may be questioned. The media which are commonly employed are the ordinary laboratory media; in addition Libman has suggested the use of serum-glucose-agar and serum-glucose-bouillon. He has pointed out that on these media the growth of most bacteria is much more marked and more rapid than on ordinary serum-agar. This is true especially of the streptococcus, the pneumococcus, the gonococcus, and the meningococcus.

Petruschky has shown that in severe cases of septic infection it is almost always possible to find streptococci in the blood, while in the

milder cases a negative result is usually reached. He has found, moreover, that while as a rule the presence of streptococci will justify a grave prognosis *quoad vitam*, death does not necessarily occur in every case. Other investigators have arrived at similar conclusions. Petruschky's positive findings include 5 cases of sepsis following phlegmonous abscess or pneumonic infection, with 3 deaths and 2 recoveries; 9 cases of puerperal infection, with 3 deaths and 6 recoveries; 1 case of ulcerative endocarditis (fatal termination); and 2 cases of mixed infection with 1 death and 1 recovery. In 15 of the 17 cases streptococci were found; in the 2 remaining cases staphylococci were present.

Lenhartz obtained positive results *intra vitam* in 16 cases of endocarditis out of 28. The organisms encountered were staphylococci, streptococci, the diplococcus pneumoniæ, and in one instance the gonococcus. Most commonly a streptococcus parvus was found. Libman states that in cases of acute ulcerative endocarditis he has always found organisms in the blood; he reports 2 instances, 1 acute and 1 chronic, in which the staphylococcus aureus was obtained in pure culture. Cole has recorded 1 instance of malignant endocarditis with septicæmia in which the staphylococcus albus was found. Libman reported a similar case, but later expressed the opinion that the organism may have been the staphylococcus aureus. He adds that in the last four years he had not met with a single instance in which he could ascribe a systemic infection to the staphylococcus albus. The same writer has further reported a series of 23 cases of systemic infection by the staphylococcus aureus, comprising a number of cases of osteomyelitis; of these 23, 5 recovered. The amount of blood used in the examinations was usually from 5 to 15 c.c., sometimes as much as 25 c.c.[1]

Pus organisms have been repeatedly found in the blood in phthisis, in advanced cases of the disease. F. Meyer and Michaelis state that they obtained positive results in 8 of 10 cases. Sittmann, Jacowsky, and Hewelke report similar findings, while others have been less successful, owing to the fact apparently that they used too little blood. Meyer and Michaelis suggest that it is not advisable to take less than 10 c.c.

Hektoen has pointed out that in scarlatina streptococci may be found in the blood during life in at least 18 per cent. of all cases; I append his conclusions: Streptococci may occasionally be found in the blood of scarlet fever cases that run a short, mild, and uncomplicated clinical course. They occur with relatively greater frequency in the more severe and protracted cases of the disease, in which there

[1] In a recent communication Libman tells me that he found attenuated streptococci in five cases of mild acute endocarditis following what clinically *appeared to be* typical articular rheumatism. They could be demonstrated during extended periods of time. He also notes that he has recently observed a case of systemic infection by the staphylococcus citreus (the first instance of its kind in his series).

may also develop local complications and clinical signs of general infection, such as joint-inflammations; but even in the grave cases of this kind spontaneous recovery may take place. In fatal cases streptococci may not be demonstrable. The theory that scarlet fever is a streptococcus disease thus does not seem to receive direct support from these investigations.

In diphtheria, measles, and smallpox infection with streptococci is also not uncommon. Other organisms may, however, also be met with, such as the various staphylococci, and quite commonly also, according to Jehle, the bacillus of influenza. He found the organism in question in 22 cases of scarlatina out of 48 that ended fatally; in measles 15 times out of 23; and in 5 cases of varicella out of 9. In Hektoen's series, on the other hand, the organism was not found; but it is noted that during the year influenza was not especially prevalent in Chicago. In the only 2 fatal cases of Hektoen's the staphylococcus aureus was found, and no streptococci.

Of other organisms which may be met with in septic conditions, the diplococcus pneumoniæ is the most common. Aside from pneumonia, it has been found in peritonitis, associated with carcinoma of the uterus, in cases of suppurative oöphoritis, following childbirth, in cases of biliary abscess at the time of the chill, etc. Friedländer's bacillus has also been found. In several cases of gonorrhœal septicæmia the gonococcus has been isolated during life (see below). Proteus vulgaris has been found in a few instances. The bacillus aërogenes capsulatus, which is so frequently seen after death, has also been obtained from the blood of living patients. Quite recently also a newly discovered micro-organism has been isolated from the blood by MacCallum and Hastings, which they term the micrococcus zymogenes. It is apparently closely related to the pneumococcus and the streptococcus pyogenes.

The number of organisms which may be found in the blood in septic conditions is exceedingly variable. On the one hand, but one plate or flask out of several may show any growth, and then only after several days; while, on the other hand, the number of organisms may be quite large. Cole has reported a case of streptococcus septicæmia in which the number of organisms amounted to 3642 per c.c. of blood six days before death, and then rose to 10,716 per c.c. two days before death.

The time before death at which organisms may be found in the blood is also quite variable; sometimes they may be demonstrable a month before, in other cases only a day or two before the fatal issue.

The *Staphylococcus pyogenes aureus* occurs in the form of spherical bodies, averaging about 0.8 μ in diameter, which readily stain with the basic anilin dyes, as also with Gram's method. They usually occur in clumps, but may also be seen in pairs and in short

chains. The organism grows on all culture-media, and in the presence of oxygen gives rise to the formation of an orange-yellow pigment. Gelatin is rapidly liquefied; it coagulates milk and clouds bouillon. The *Staphylococcus pyogenes albus* and *citreus* differ from the aureus by the absence of pigment in the first and by the formation of a lemon-yellow pigment in the second.

The *Streptococcus pyogenes* (Plate VII., Fig. 1) occurs in chains of spherical cocci which usually vary from four to twenty in number. The size of the individual organism is somewhat greater than that of the staphylococcus, but may vary even in one and the same chain. It is readily stained with the basic anilin dyes and also with Gram's method. It grows on all culture-media at the temperature of the room, forming small gray granular colonies on agar and gelatin. As a rule, it does not liquefy gelatin, and it may or may not coagulate milk and cloud bouillon. Several varieties are recognized, viz., *Streptococcus brevis*, which forms short chains; *Streptococcus longus*, which occurs in long chains; streptococci which render bouillon cloudy, and those which do not; streptococci which form flocculent, sandy, scaly, or viscous sediments.

The *Streptococcus conglomeratus* grows, without clouding bouillon, in the form of dense separate particles, scales, or thin membranes at the bottom and sides of the tube, and on shaking the sediment it breaks up into little specks, without producing uniform, diffuse cloudiness. The chains are long and interwoven in conglomerate masses (Welch).

Literature.—F. W. White, "Cultures from the Blood in Septicæmia, Pneumonia, Meningitis, and Chronic Diseases," Jour. Exper. Med., vol. iv. p. 425. Petruschky, Zeit. f. Hyg., vol. xvii. p. 59. Sittmann, Deutsch. Arch. f. klin. Med., vol. liii. p. 323. Canon, Deutsch. Zeit. f. Chir., vol. xxxiii. p. 571; and Mitth. aus d. Grenzgeb. d. Med. u. Chir., 1902, vol. x. p. 41. Lenhartz, Münch. med. Woch., 1901. Nos. 28 and 29. Libman, Proc. N. Y. Path. Soc., 1903, vol. iii. pp. 5 and 57; "On Certain Features of the Growth of Bacteria," etc., Jour. Med. Research, 1901, vol. vi. Cole. Johns Hopkins Hosp. Bull., 1902, vol. xiii. p. 252. Wm. Welch, "Morbid Conditions Caused by the Bacillus Aërogenes Capsulatus," Ibid., 1899, vol. x. p. 134. Gwyn, Ibid., 1900, vol. xi. p. 185 (first case); Cole, Ibid., 1902, vol. xiii. p. 234 (second case). Hektoen, Jour. Am. Med. Assoc., 1903, vol. xl. No. 11. Jehle, Zeit. f. Heilk., 1901, vol. xxii. p. 190. Ewing, Trans. Am. Assoc. Phys., 1902, vol. xvii. p. 208.

Gonococcus Septicæmia.

Cases of gonorrhœal septicæmia in which the gonococcus was isolated from the blood of the patients during life have been reported by Thayer-Blumer, Thayer-Lazear, Byelogoway, Wilson, and Harris-Johnston. In all these cases gonorrhœal endocarditis existed. In other infections of the same nature positive results were obtained by Ahmann, Colombini, Panichi, and Unger, in association with polyarthritis, epididymitis, myositis, tenovaginitis, inguinal bubo, and parotitis. In the endocarditis cases cultures were obtained after an illness lasting for from five weeks to seven months, at times as early

PLATE X

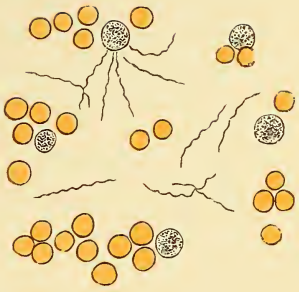
FIG. 1.

Streptococcus Pyogenes. (Abbott.)

FIG. 2.

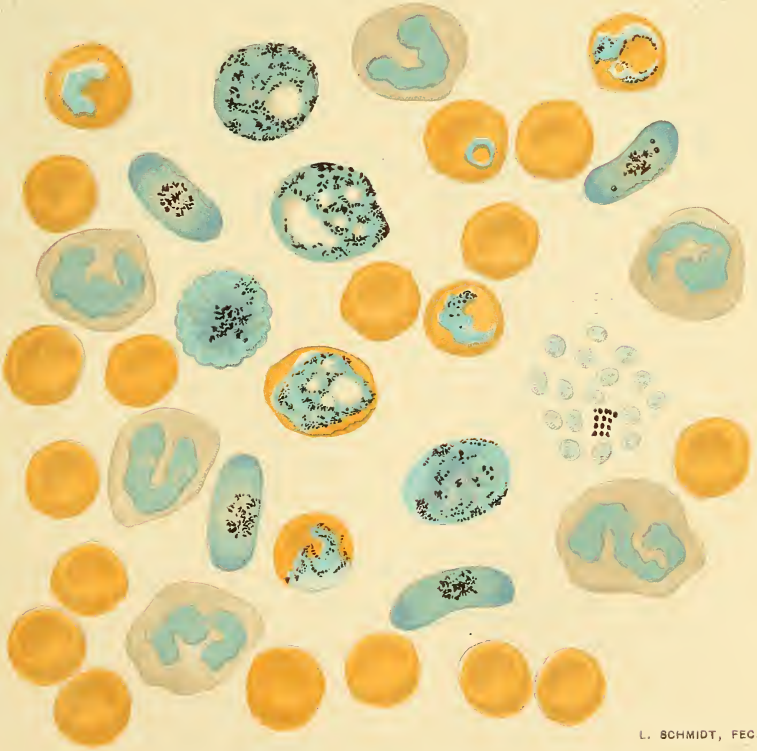
Bacillus Anthracis, highly magnified to show Swellings and Concavities at extremities of the Single Cells. (Abbott.)

FIG. 3.



Spirilla of Relapsing Fever. (v. Jaksch.)

FIG. 4.



Malarial Blood Stained with Chenzinsky-Plehn's Solution. (Personal Observation.)

as the ninth to the eleventh day preceding death, and on an average five days before death.

To cultivate the gonococcus from the blood during life, it is neither necessary to use a large amount of blood nor to dilute it greatly, nor to employ any specially prepared medium. From 2 to 5 c.c. are sufficient. According to Harris and Johnston, it is more advantageous to mix the blood with melted agar and to plate the same, than to use fluid media where the oxygen supply is more restricted.

For a description of the organism, see page 644.

LITERATURE.—N. M. Harris and W. B. Johnston, "Gonorrhœal Endocarditis with Cultivation of the Specific Organism from the Blood during Life," Johns Hopkins Hosp. Bull., 1902, vol. xiii. p. 236 (literature). Thayer and Blumer, Ibid., 1896, vol. vi. p. 59. Thayer and Lazear, Jour. Exper. Med., vol. iv. p. 81.

Anthrax.

The bacillus of anthrax, as first pointed out by Pollender, Brouell, and Davaine, is frequently met with in the blood, where it should be sought for in doubtful cases by staining with Löffler's method. The number of the organisms present, however, is probably always small. Cover-glass preparations are floated for five to ten minutes on a mixture of 30 c.c. of a concentrated alcoholic solution of methylene-blue and 100 c.c. of a 1 : 10,000 solution of potassium hydrate; they are then washed for five to ten seconds in a 0.5 per cent. solution of acetic acid, treated with alcohol, dried, and mounted in balsam. Thus stained, the bacilli appear as rods measuring from 5 μ to 12 μ in length by 1 μ in breadth, and usually present a segmented appearance, the extremities being slightly thickened. Spores are not found, as the organism multiplies by fission. When present in large numbers it is not even necessary to stain, as the organisms can then be seen without difficulty in fresh specimens (Plate X., Fig. 2).

In doubtful cases, in which a microscopical examination of the blood yields negative results, a few cubic centimeters of the blood may be injected into a mouse or a guinea-pig, in the blood of which the bacilli will soon be found in enormous numbers if the disease is anthrax.

LITERATURE.—Pollender, Casper's Vierteljahrsch. f. gerichtl. u. öffentl. Med., 1855, vol. viii. p. 103. Brauell, Virchow's Archiv, vol. xi. p. 132, and vol. xiv. p. 32. Davaine, Compt. rend. de l'acad. d. sci., vol. lvii. p. 220. Blumer and Young, Johns Hopkins Hosp. Bull., 1885, p. 127.

Acute Miliary Tuberculosis.

In acute miliary tuberculosis tubercle bacilli have repeatedly been observed in the blood; but while their presence may be regarded as pathognomonic of the disease, the search for them is most tedious and often in vain. Nevertheless a careful examination of the blood is indicated in doubtful cases; but only a positive result is of value.

According to Liebmann, the tubercle bacilli are most numerous in the blood about twenty-four hours after the injection of tuberculin. Working in this manner, he claims to have obtained positive results in 56 cases of 141. As a rule it is probably better to resort to the animal experiment.

For methods of staining and a description of the tubercle bacillus, the reader is referred to the chapter on Sputum.

LITERATURE.—Liebmann, Berlin. klin. Woch., 1891, p. 393. Krönig, Deutsch. med. Woch., 1894, vol. v. p. 42.

Glanders.

In glanders the specific bacillus is constantly present in the blood, and may be demonstrated by staining the dried preparations on a cover-glass for five minutes with a concentrated alcoholic solution of methylene-blue, mixed with an equal volume of a 1 : 10,000 solution of potassium hydrate just before using. From this mixture the specimen is passed for a second or two into a 1 per cent. solution of acetic acid which has been tinged a faint yellow by the addition of a little tropæolin 00 solution; it is then decolorized by washing in water containing 2 drops of concentrated sulphuric acid

FIG. 34.

Bacillus of glanders. (ABBOTT.)

and 1 drop of a 5 per cent. solution of oxalic acid for each 10 c.c. In specimens thus stained, the bacilli appear as short rods measuring from 2 μ to 3 μ in length by 0.3 μ to 0.4 μ in breadth, often containing a spore at one end (Fig. 35).

LITERATURE.—Duval, Arch. de méd. expér., 1896, p. 361.

Influenza.

In the sputum of influenza a specific organism has been described by Pfeiffer and Kitasato; it is said to be constantly present also in the blood of such patients. The organism in question appears in the form of minute rods measuring 0.1 μ in breadth by 0.5 μ in length occurring either singly or in chains of three or four. In suitably prepared specimens, owing to the fact that their poles take up the stain more readily than the middle portion, they convey the impression of diplococci.

Canon advises the following method for demonstrating their presence in the blood: cover-glass preparations that have been allowed to dry at ordinary temperature are placed in absolute alcohol for five minutes and are then stained at a temperature of 37° C. for from three to six hours, with Chenzinsky-Plehn's solution (see page 101). The specimens are washed in water, dried between layers of filter-paper, and mounted in balsam. Stained in this manner, the red corpuscles are colored red, and the leucocytes, as well as the bacilli, blue. As a rule, only from four to twenty are found in one preparation, usually occurring singly, but also in groups. Owing to the fact that they are found in the blood only during the acme of the disease, Canon recommends examination of the sputum for diagnostic purposes, a view with which my own observations are entirely in accord. Some observers indeed deny the occurrence of the organism in the blood altogether (Kühnau).

LITERATURE.—Canon, Virchow's Archiv, vol. cxxxi. p. 401. Klein, Baumgarten's Jahresb., 1893, p. 206. Kühnau, Zeit. f. Hyg., vol. xxv. p. 492.

Relapsing Fever.

Relapsing fever is characterized by the presence in the blood, and here only, of spirilla or spirochætæ which bear the name of their discoverer, Obermeier. In order to search for these organisms no special precautions are necessary. After having carefully cleansed the finger, as described, a drop of blood is mounted on a very thin cover-glass. This is inverted directly upon the slide, when the specimen is ready for examination; an oil-immersion lens is not required. Attention is drawn to the presence of the organisms by certain disturbances which are noticeable among the red corpuscles, and upon careful examination it will be seen that these are caused by the wriggling movements of the spirilla. The Spirochætæ Obermeieri are long, slender filaments, measuring from 36 μ to 40 μ in length by 0.3 μ to 0.5 μ in breadth, and present from eight to twelve incurvations of equal size with tapering extremities (Plate X., Fig. 3). These two last characteristics serve to distinguish this species from that described by Ehrenberg, in which the radius of the incurvations is not the same in all, and in which the extremities do not taper.

The number of spirilla which may be found in a drop of blood varies, being greater during the access of the fever, when twenty, or even more, may be observed in the field of the microscope. They occur singly or in bunches of from four to twenty, specimens resembling those figured in the illustration being frequently seen. In the quiescent stage they are arranged sometimes in the form of rings or of the figure 8. After the crisis they seem to disappear entirely, and their presence during an afebrile period may therefore be regarded as indicating a pseudocrisis. During the afebrile periods small, bright,

round bodies have been described as occurring in the blood, which according to some are spores, but according to others represent merely débris of the spirilla.

Culture-experiments have not been very satisfactory, although Koch observed an increase in their number at a temperature of from 10° to 11° C.

That confusion should ever arise in distinguishing the spirilla of relapsing fever from the free flagella observed at times in malarial blood seems to me very improbable.

LITERATURE.—Heidenreich. Untersuch. über d. Parasit. d. Rückfallstyphus, Berlin, 1877. Moczutkowsky, Deutsch. Arch. f. klin. Med., vol. xxiv. p. 80, and vol. xxx. p. 165. Blisener, Inaug. Diss., Berlin, 1873. Engel, Berlin. klin. Woch., 1873, p. 409.

Malta Fever.

In Mediterranean or Malta fever the specific organism, the *Micrococcus melitensis* (Bruce), has been isolated from the blood during life, but as a rule the findings are uncertain. Diagnosis is facilitated by the fact that a well-pronounced agglutination is obtained with the patient's serum. A positive reaction with a dilution of more than 1 : 20, according to Birt and Lamb, may be regarded as proof positive of the existence of the disease. As a rule agglutination can still be obtained with a dilution of from 1 : 600 to 1 : 700. It begins about the fifth day of the disease, and gradually diminishes in intensity during convalescence, but may persist for a year and a half, and even longer.

The organism in question is a coccus, measuring 0.3 μ in diameter, and occurs singly, in pairs, and sometimes in fours. Longer chains are not seen. It is motile. It is stained by the usual dyes and grows best on 1.5 per cent. of very feebly alkaline, peptonized agar-beef jelly. After thirty-six hours the colonies are a transparent amber, while later they are opaque. Liquefaction does not occur.

LITERATURE.—C. Birt and G. Lamb, "Mediterranean Fever," Lancet, Sept. 9, 1899. Wright and Smith, Brit. Med. Jour., April 10, 1897. Musser and Sailer, Phila. Med. Jour., 1898, p. 1408, and 1899, p. 89. R. P. Strong and W. E. Musgrave, "The Occurrence of Malta Fever in Manila," Phila. Med. Jour., 1900, p. 996. J. J. Curry, "Malta Fever," Jour. Med. Research, July, 1901.

Yellow Fever.

Wasdin and Geddings, constituting a commission of medical officers of the U. S. Marine-Hospital Service detailed by the U. S. government to investigate the cause of yellow fever, report that Sanarelli's bacillus may be isolated from the blood of the patients during life. They found the organism in twelve cases out of fourteen after the third day of the disease, and also obtained it from the remaining two after death. In other diseases it was not found.

A similar commission, consisting of Reed, Carroll, Agramonte,

and Lazear, on the other hand, arrive at negative results. By withdrawing the blood from the veins of nineteen patients they failed to obtain a positive result in every instance. Post-mortem investigations in eleven cases were likewise negative.

According to Reed and Carroll, Sanarelli's Bacillus icteroïdes should be considered a variety of the hog cholera bacillus, and as a secondary invader in yellow fever.

Infection occurs through the bite of mosquitoes (Culex fasciatus, Fabr., and probably other varieties also) which have previously fed on the blood of yellow fever patients. The period after contamination which must elapse before the mosquito is capable of conveying the infection averages twelve days in summer, and eighteen or more days during the winter months.

LITERATURE.—"Controversy between G. Sanarelli and W. Reed and J. Carroll on the Specific Cause of Yellow Fever," Med. News, 1899, pp. 193, 321, 513, and 737. E. Wasdin and H. D. Geddings, Report of Commission of Medical Officers to Investigate the Cause of Yellow Fever, Treasury Dept., U. S. Marine-Hospital Service, 1899. Reed, Carroll, and Agramonte, Jour. Am. Med. Assoc., 1901, p. 431.

Bubonic Plague.

In advanced cases of bubonic septicæmia the specific organism may be found in the blood in small numbers. Toward the end of rapidly fatal cases they become more numerous, and may then be demonstrable directly with the microscope.

The organism in question, the *Bacillus pestis* (Kitasato, Yersin), is a short, thick cocco-bacillus, with rounded ends, measuring about 2 μ in its greatest diameter. Examined in the hanging drop it is devoid of automobility. The polar regions are readily stained, while the interpolar area remains colorless. In many organisms a capsule can be made out by appropriate methods, but it is apparently not a constant feature. Oftentimes the form of the organism deviates from the normal. It may thus resemble a coccus on the one hand, while on the other it appears more elongated. It is decolorized by Gram's method.

The blood-smears are fixed by immersion in absolute alcohol for twenty-five minutes; or they are covered with absolute alcohol for about one-half minute, when the alcohol is burned off. For staining purposes, borax methylene-blue (a solution of 2 per cent. methylene-blue in 5 per cent. borax-water) or Löffler's alkaline methylene-blue may be conveniently employed. In the first instance we stain for one-half minute, in the second for from two to three minutes. The polar staining is in this manner quite satisfactory.

On gelatin-agar containing 2.5–3.5 per cent. of salt and in bouillon a fairly characteristic growth results. In the case of the agar involution-forms are obtained, among which long, slender

bacilli, which are segmented and present a vacuolated appearance, are especially noteworthy. In this state they stain quite badly and have lost a certain degree of their virulence. In bouillon the organism often forms long chains of well-rounded bodies which are quite similar to a coccus. During its growth in bouillon it forms flakes or flocculi, which rapidly sink to the bottom of the tube, leaving the liquid clear above. Colonies on gelatin about thirty-six hours old are warty, strongly refractive formations, which often present a delicate, irregularly indented margin. Even after twenty-four hours one can obtain smears, in which 50–100 bacilli are grouped in little colonies of irregular form, while examination of the plates with a magnifying power of 60 diameters reveals scarcely any growth. The organism does not liquefy the gelatin. The optimum temperature for growth is between 36° and 39° C.

LITERATURE.—For Kitasato's report see: Annual Rep. of the U. S. Marine-Hospital Service for 1894; W. Wyman, Bubonic Plague; U. S. Treasury Dept., 1900. Kossel u. Overbeck, Arb. aus d. Kais. Gesundheitsamt., 1901, vol. xviii.

Malaria.

The discovery in the blood of a specific micro-organism belonging to the class of protozoa, the *Plasmodium malariæ* of Laveran, and its invariable presence in the different forms of this disease, must be regarded as one of the most important in clinical medicine. This is not the place to state how frequently a diagnosis of malarial fever based upon clinical symptoms alone has proved false, nor how often a tubercular, a syphilitic, or a septic infection has been overlooked and termed malaria. It will suffice to say that errors of this kind, in view of our present knowledge and the ease with which they can be avoided by every physician, should no longer occur. *The diagnosis of malaria should in every case be based upon a microscopical examination of the blood.* The search for the specific organism, it is true, may be very tedious at times, but it will always be crowned with success if the disease in question is malaria.

The parasite in question, as I have stated, is a protozoön, and belongs to the class of hæmatozoa, representatives of which are found in the blood of various animals, such as the rat, frog, turtle, carp, various birds, etc. Three varieties are known to occur in the blood of man, viz., the parasite of tertian, quartan, and æstivo-autumnal fever. The life-history of these organisms is now well understood, and it is known that in addition to the intra-corporeal cycle of development which takes place in the human body there is yet another, an extra-corporeal cycle, which occurs in certain mosquitoes of the genus Anopheles. Infection occurs through the bites of such mosquitoes, which themselves have been infected by sucking the blood of malarial patients. This has been abundantly

demonstrated by Ross, Manson, Grassi, and others, and may be regarded as an established fact.

Method of Examination.—The necessary amount of blood is best obtained by puncture of a finger or the lobe of the ear. Cover-glass specimens are then prepared as usual and may be examined either wet or stained with one of the modifications of the Romanowsky method, or the eosinate of methylene-blue (see page 127).

Ross has recently suggested the advisability of spreading thick blood specimens and extracting the hæmoglobin before staining for the malarial organisms, when these are only present in small numbers. The search for the youngest forms of the æstivo-autumnal parasite especially is in this manner much facilitated. Ruge endorses this method in the following modification, but points out that the specimens are by no means beautiful owing to precipitation of pigments. A large drop of blood (about 20 cbmm.) is spread over a surface measuring about 18 square millimeters. The air-dried preparation is then placed for a few minutes in a 5 per cent. solution of formalin,[1] to which 0.5–1 per cent. of acetic acid has been added. In this manner the hæmoglobin is all extracted, while at the same time the blood-film is fixed, so that it can now be washed without fear of ruining the preparation. This is then stained either according to one of the modifications of the Romanowsky method, or with the eosinate of methylene-blue, carbol-thionin, etc. Ruge further advises that specimens stained according to the Romanowsky method be subsequently stained with Manson's solution,[2] in order to render the smallest and medium-sized ring-forms more readily visible, as their affinity for the dye is somewhat impaired by the fixation in formalin.

The Parasite.—The following forms of the parasite may be found in the blood:

1. Hyaline Non-pigmented Intracellular Bodies.—These apparently represent the earliest stage in the development of the parasite, and are found in all forms of malarial fever; they are especially abundant during the latter part of the paroxysm or immediately thereafter. At first sight they may be mistaken for vacuoles, but upon closer examination it will be found that they exhibit distinct movements of an amœboid character, and may thus easily be recognized with a little experience.

The rapidity with which these changes in the form of the organism occur in the tertian type of ague is most astonishing, and sketches of any one phase can often, indeed, be made only from memory;

[1] This solution would contain 2 per cent. of formaldehyde gas, as the commercial formalin is about a 40 per cent, solution.

[2] This is an aqueous solution of borax (5 per cent.) and methylene-blue (2 per cent.). The blood-films are stained with this solution for about thirty seconds; they are then washed in water, dried with filter-paper, and afterward by gently warming them over the flame.

in quartan fever the movements are much slower and far less extensive.

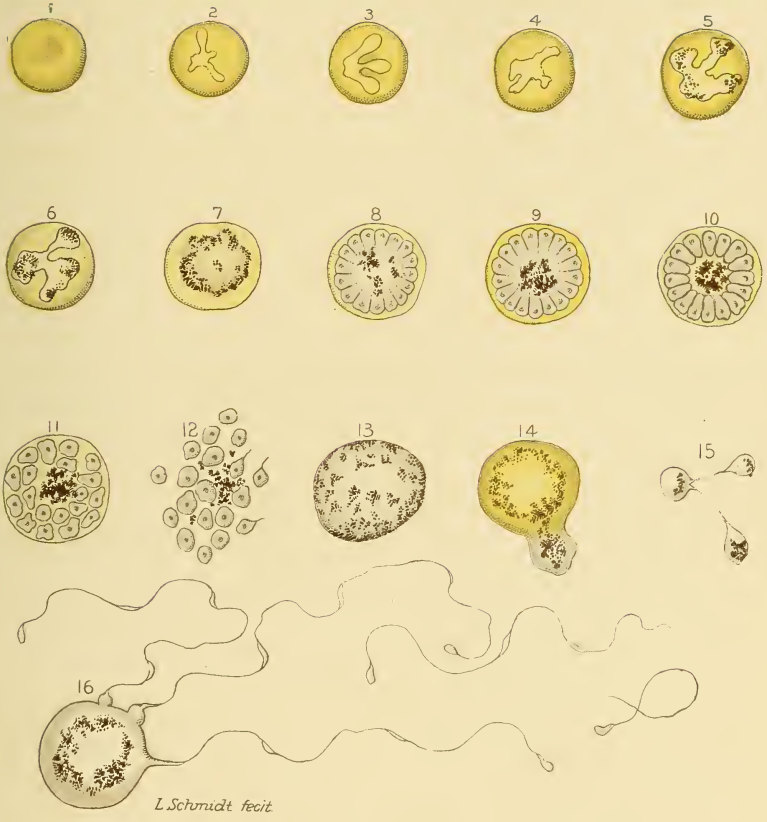
In the irregular fever of the æstivo-autumnal form amœboid movements may likewise be observed, but more commonly the parasite assumes a ring-like appearance, and does not throw out distinct pseudopodia. If these forms are carefully observed, however, it will be found that they are not absolutely quiescent, but alternately expand and contract.

In tertian fever the organism (Plate XI.) is pale and indistinct, while in quartan fever it is sharply outlined and somewhat refractive (Plate XII., Fig. 2). In the æstivo-autumnal form the organism is usually much smaller than in the tertian type, and the ring-like bodies frequently present at some point in their interior a distinctly shaded aspect which closely resembles the darker portion in the centre of a normal corpuscle (Plate XII., Fig. 1). It is thus possible, even at this stage in the development of the parasite, to distinguish between fever of the tertian, quartan, and æstivo-autumnal type.

The numbers in which these small, non-pigmented intracellular organisms may at times be met with is most astonishing. In a case of pernicious malarial fever of the algid type, which I had occasion to examine, and in which a history of only one week's illness without chills was obtained, normal red corpuscles were indeed only exceptionally found. The case was one of the æstivo-autumnal form of fever.

2. Pigmented Intracellular Organisms.—These represent a later stage in the development of the parasite, and, like the non-pigmented intracellular bodies, are met with in all types of malarial fever. Their appearance, however, differs considerably in the various forms. In tertian fever minute granules of a reddish-brown color appear in the bodies of the organism very soon after the paroxysm. These gradually increase in number, while the invaded corpuscles proportionately become paler and paler, until finally only an indistinct, shell-like outline can be discerned. In fresh specimens the granules, which often assume the form of little rods, resembling bacteria, exhibit most active molecular movements, attracting attention at once. The body of the parasite, which during its development has increased gradually in size, is probably hyaline, and may still be seen to undergo amœboid movements. These are not nearly so active, however, as in the non-pigmented stage. The movements, moreover, cannot be followed so readily, owing to the presence of the granules. At first sight, these appear to be scattered in small collections throughout the red corpuscle, and the impression may be gained that several organisms are present at the same time. Upon closer investigation, however, it will be seen that this is only apparently the case, and that the granules are confined to the bulb-

PLATE XI.

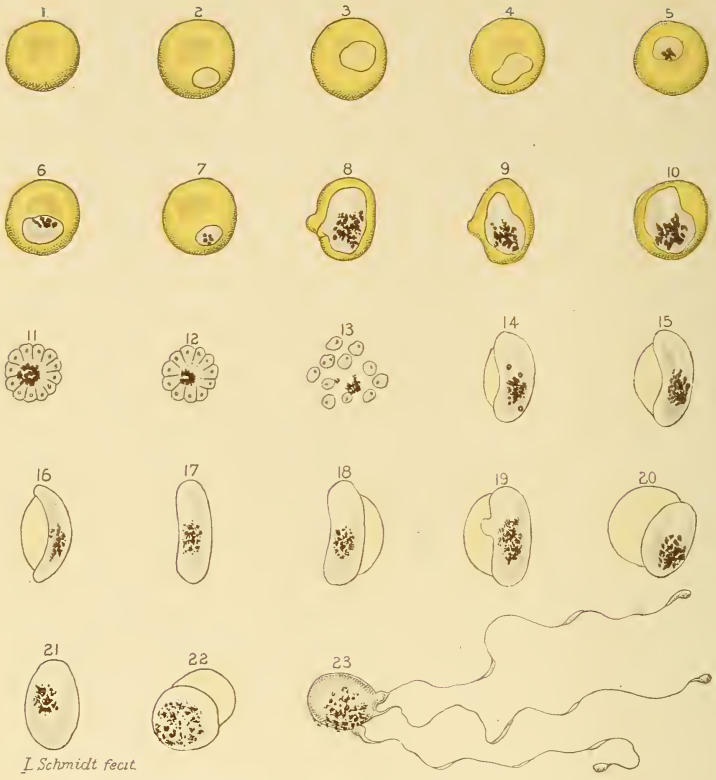


The Parasite of Tertian Fever.

1, Normal Red Corpuscle; 2-4, Non-pigmented Stage of the Organism, showing Amœboid Movements; 5-7, Progressive Pigmentation and Growth; 8-11, the Process of Segmentation; 12, Young Forms; 13, Large Extra-cellular Organism; 14, Mode of Formation of Extra-cellular Body; 15, Small Fragmented Extra-cellular Organism; 16, Flagellate Body and Free Flagella. Unstained Specimen. (Personal Observation.)

PLATE XII.

FIG. 1.



The Parasite of Aestivo-Autumnal Fever.

1, Normal Red Corpuscle; 2-10, Gradual Growth of the Organism; 11 and 12, Segmenting Bodies; 13, Young Forms; 14-22, Crescents, Ovoids and Spherical Bodies, with and without Bib; 23, Flagellate Body. Unstained Specimen. (Personal Observation.)

FIG. 2.

The Parasite of Quartan Fever.

1, Normal Red Corpuscle; 2-6, Gradual Growth of the Organism; 7, Pigmented Extra-cellular Body; 8, Segmenting Body; 9, Young Forms; 10, Vacuolated Extra-cellular Body; 11, Flagellate Form. Unstained Specimen. (Personal Observation.)

ous extremities of the pseudopodia of a single parasite. Before the end of forty-eight hours the organism has filled out the entire red corpuscle, which at the same time has attained a larger size than normal. The amœboid movements become less and less marked, and the pigment-granules, which may still be quite active, tend to collect about the periphery (Plate XI.).

In quartan fever pigmented intracellular bodies likewise appear very soon after the paroxysm. The individual granules, however, are somewhat larger, of more irregular size, and darker in color than those seen in the tertian type (Plate XII., Fig. 2). Instead of exhibiting active molecular movements, moreover, they are almost entirely quiescent, and usually are grouped along the periphery of the organism. While amœboid movements can at first be observed, these become less and less marked, until finally, at the end of from sixty-four to seventy-two hours, they cease. The organism then presents a round or ovoid form, but does not fill the red corpuscle entirely. It is curious to note that in this form of ague the red corpuscles do not become decolorized, but rather darker than normally, and at times specimens may be seen which present a distinctly greenish or brassy appearance. When the parasite has become fully developed the corpuscle is smaller than normally, and, on staining, it may be seen that the organism still is surrounded by a narrow zone of corpuscular protoplasm even when this is not apparent in unstained preparations.

The pigmented intracellular bodies which may be found in æstivo-autumnal fever (Plate XII., Fig. 1) can readily be distinguished from those observed in tertian and quartan ague. As in these types, pigment-granules also appear after the paroxysm; they are never numerous, however, and often only one or two minute dark granules can be detected near the periphery. The organism, even in the later stages of its development, scarcely ever occupies much more than one-third of the corpuscle. Usually the granules exhibit scarcely any movements. As in the quartan type of ague, decolorization of the red corpuscles does not occur, and here, as there, a greenish, brassy appearance often is observed. At times the red corpuscles are shrunken, crenated, or spiculated.

At the beginning and during the paroxysm forms are at times seen in which the few pigment-granules that may be present have gathered in the centre of the parasite and formed a solid clump. From the facts that these are observed only during the paroxysm, and that central blocks of pigment are found only during the stage of segmentation (see below) in tertian and quartan ague, Thayer and others conclude that these bodies are pre-segmenting forms of the parasite. This belief is strengthened further by the observation that pigment-bearing leucocytes are then also seen, which in the other types of fever likewise are found only at this time.

3. Segmenting Bodies.—In cases of tertian and quartan fever the process of segmentation may be observed directly under the microscope, if specimens of blood are obtained just prior to or during the chill. In tertian fever organisms will then be seen in which the destruction of the red corpuscles has advanced to a stage in which it is only possible to make out a pale contour of the original host. The parasite itself has assumed gradually a granular appearance, and the pigment-granules, which until then have exhibited pronounced molecular movements, now become quiescent, larger and rounder, and show a distinct tendency to collect in the centre of the body. Here they form a roundish mass in which the individual components can scarcely be made out. While this change in the position of the pigment is taking place, beginning segmentation of the surrounding granular protoplasm will be observed. This at first is most marked at the periphery, from which delicate striæ will gradually be seen to extend toward the central mass, dividing up the protoplasm into a number of oval bodies which closely resemble the petals of a flower (Plate XI.). Still later these bodies, which in reality are the sporules of the parasite, will be found scattered in an irregular manner throughout the interior of the organism. The apparent envelope then disappears, and the sporules, which in tertian fever usually number from fifteen to twenty, lie free in the blood. Quite frequently, also, a sudden expulsion of the little bodies is observed and the impression gained as though the envelope had been burst asunder. Upon closer inspection, even at the petal stage, it will be seen that almost every sporule presents a tiny dot in its interior, which may at first sight be mistaken for a pigment-granule, but which in all probability is a nucleus. After the expulsion of the sporules these are frequently seen to move about in an active manner, but sooner or later they come to rest.

While the progress of segmentation is very frequently observed to proceed in the manner described, this is not invariably the case. It may thus happen that segmentation occurs before the pigment-granules have had time to gather at the centre, or that the parasitic protoplasm breaks up into sporules directly without the intervention of the petal stage. In every case, however, the formation of sporules is associated directly with the occurrence of a paroxysm, and represents the asexual type of reproduction of the parasite.

The ultimate fate of the sporules is not definitely known, but it is likely that they in turn invade new corpuscles, cause their destruction, and become segmented, thus giving rise to a new generation. As the process of segmentation, moreover, coincides in time with the occurrence of the chill, it is apparent that the interval elapsing between two consecutive chills—*i. e.*, the type of the ague—depends upon the rapidity with which the non-pigmented forms arrive at maturity.

In quartan ague the manner in which segmentation takes place differs somewhat from that observed in the tertian form. It will here be observed that the pigment-granules, which have gathered along the periphery of the organism, as the parasite approaches maturity become arranged in a stellate manner, and apparently reach the centre through definite protoplasmic channels. Here they finally form a dense clump, and while the protoplasm assumes a finely granular appearance, segmentation proper begins and proceeds as in the tertian form. In quartan ague, however, the number of segments is smaller, varying between six and twelve. The entire segmenting body, moreover, is smaller than in the tertian form, and the segments are arranged in a more symmetrical manner. Here, indeed, the most perfect rosettes are observed (Plate XII., Fig. 2).

In æstivo-autumnal fever segmenting bodies are only exceptionally seen in the peripheral blood, and it appears that the process of reproduction occurs principally in the spleen. The pre-segmenting forms described here undergo segmentation in a manner closely resembling that observed in tertian fever. The number of segments, moreover, is about the same, varying, as a rule, between ten and twenty. The segmenting body itself, however, is much smaller than in either the tertian or quartan form, and it is not possible to distinguish any remains of the original host.

4. Crescentic, Ovoid, and Spherical Bodies (Plate XII., Fig. 1).—These are observed only in cases of æstivo-autumnal fever when this has persisted for at least one week. At first sight they apparently bear no relation to the other forms which have been described, and it has long been a question whether or not these bodies actually represent a stage in the life-history of the common malarial parasites. Grassi and Feletti have applied the name *Laverania malariæ* to this form. More recent investigations have rendered it probable that they are derived directly from the pigmented intracellular forms. Specimens may thus be met with in which crescentic bodies are found in the interior of red corpuscles that have lost but little of their original color. Such observations, however, are not common. The typical crescents which are usually seen are highly refractive bodies, somewhat larger than a red corpuscle, measuring from 7 μ to 9 μ in length by 2 μ in breadth. Their extremities are usually rounded off and joined by a delicate, curved line bridging over their concave border. This is supposed to represent the remains of the original host. At other times this hood-like appendage is found along the convex border. The little pigment-granules and rods, which are always found in the interior of the crescents, are generally collected about the centre of the body, but they are occasionally also seen in one of the horns. While usually quiescent, a migration of some of the granules toward one extremity and back to the central mass may at times be observed.

The ovoid and spherical bodies, which are usually much smaller than the crescents, exhibit the same general features, however, and often are provided likewise with a little hood. It is now known that the spherical bodies develop from the ovoids, and these again from the crescents. Like the crescents, the ovoid and spherical forms may be found in the interior of red corpuscles.

5. Extracellular Pigmented Bodies.—In tertian and quartan ague some of the pigmented intracellular bodies, instead of undergoing segmentation when they have arrived at maturity, may be seen to leave their hosts and to appear as such in the blood. At the same time they increase considerably in size, and in the tertian form may indeed become as large as a polynuclear leucocyte (Plate XI.). The pigment-granules, moreover, exhibit an activity in their movements which is most astonishing and never observed under other conditions. The outline of the parasite is then usually irregular and quite indistinct. Upon careful observation it will be seen that in some of these bodies the movements of the granules after a while become less and less marked, and finally cease, while the body of the parasite itself becomes still more irregular in outline. This appearance is undoubtedly referable to the death of the organism. In others a gradual fragmentation is observed, small particles of the pigmented mother-substance being cut off from the parent-form. It is thus quite common to see the original parasite break up into four or five smaller bodies, in which the movements of the pigment-granules persist for some time. Sooner or later, however, even these cease, the outlines of the bodies become more and more indistinct, and death occurs. In still others the formation of vacuoles may be observed, the pigment-granules at the same time becoming quiescent. This process is likewise regarded as one of degeneration. Most interesting, however, is the fact that *flagellation* may occur in some of these extracellular forms. It will then be observed that the pigment-granules which exhibit a most surprising activity tend to collect near the centre of the organism, while at the same time curious undulating movements may be made out along its contours. Suddenly one or more (one to six) extremely slender filaments will be seen to protrude from as many points on the periphery, presenting minute enlargements here and there in their course (Plate XI.). The length of these filaments, or flagella, as they are termed, varies considerably. As a rule, it does not exceed the diameter of from five to eight red corpuscles, but much longer specimens are at times observed, and it appears to me that in most illustrations they are represented too short. With these flagella the organism makes most active whipping movements, scattering the red corpuscles to the right and left. Attention is, indeed, usually first drawn to the presence of these bodies by the disturbance which they cause in the field of vision. Occasionally one of

the flagella may be seen to become detached from the body of the parasite and to move rapidly about among the corpuscles in a snake-like manner. In microscopical specimens they gradually come to a rest and often curl into a spiral. That difficulty should ever arise in distinguishing such detached flagella from the spirilla of relapsing fever seems very improbable, as the nature of these formations is shown by the presence or absence of other forms of the malarial organism.

Beyond the fact that the flagellate organisms in tertian fever are larger than in the quartan form, no special points of difference exist (Plate XII., Fig. 2).

In æstivo-autumnal fever similar changes may be observed. In crescents it is thus not at all uncommon to observe a small hyaline protrusion from the surface of the organism, which later may become detached. This process was formerly regarded as one of regeneration, but it is questionable whether this is actually the case. In other specimens, again, true fragmentation, or vacuolization, may occur, and flagellate bodies are met with in this type of fever as well as in tertian and quartan ague. The flagellates, as in quartan fever, are smaller than those observed in the tertian form, but other points of difference do not exist (Plate XII., Fig. 1).

The significance of the flagellate organisms has until recently not been understood, but we now know that they represent the male element in the sexual reproduction of the malarial parasite, and the beginning of a cycle of development, which takes place outside of the human body, in the bodies of certain mosquitoes. The beginning of this cycle was observed first by MacCallum in the blood of infected crows. He here discovered that when one of the flagella broke loose it almost always sought out another full-grown form of the parasite which had not undergone segmentation, and penetrated this, just as the spermatozoon penetrates the ovum. Subsequently he observed the same process in the blood of the human being, which has since been confirmed by others. The further development of the fertilized forms, however, does not take place in the human blood, but in the bodies of mosquitoes. The fertilized organism then penetrates the stomach-wall of the insect, and here gives rise to the formation of little cysts, in which after about seven days numerous irregular, rounded, ray-like striæ appear. After a time the capsules of the cysts burst, and the delicate, thread-like bodies (the sporozoites) are set free in the body cavity of the mosquito, and shortly after appear in the salivary glands. These bodies apparently represent the young parasites, which result from the sexual reproduction of the adult organism. If at this stage of their development the infested mosquito is allowed to bite a human being, malarial infection results, with the appearance in the blood of the hyaline forms already described.

From the above description it will be seen that three forms of the malarial parasites may be found in the blood, viz., the parasite of tertian, quartan, and æstivo-autumnal fever, and it has been shown that these forms may readily be distinguished from each other. It should be mentioned, however, that in tertian and quartan fever several groups of the same organism may be present at one time, and as the process of segmentation coincides with the occurrence of a paroxysm, it will readily be seen that the number of paroxysms within a given time depends directly upon the number of groups which may be present in the blood. If a double infection with the tertian parasite has occurred, one group of organisms may thus have just reached the segmenting stage, while the second group has attained only a twenty-four hours' growth, the result being that maturity is reached by the two groups on successive days. Quotidian fever is then the result. Should still other groups be present, the clinical picture will accordingly become more complicated. In quartan ague, similarly, double quartan fever will occur if two groups are present, and triple quartan fever if three groups are present at one time. Mixed infections, further, are also possible.

In conclusion, it may not be out of place to refer to the presence of pigment-bearing leucocytes in the blood of malarial patients. These are quite constantly met with during the paroxysm, and it is indeed often possible to observe the process of *phagocytosis* directly under the microscope (see Fig. 15). The forms which are taken up are the central pigment-clumps of organisms that have undergone sporulation, the small, fragmented extracellular forms, the flagellate bodies, and even the segmenting bodies. In every case where pigment-bearing leucocytes—which are probably always of the neutrophilic, polynuclear variety—are observed malarial fever should be suspected and a careful examination made, as a melanæmia has so far been observed only in this disease, in relapsing fever, and in connection with the rare melanotic tumors, in which not only leucocytes containing melanin occur in large numbers, but also masses of this pigment float free in the blood.

LITERATURE.—A. Laveran, Nature parasitaire des accidents de'limpaludisme, Description d'un nouveau parasite, Paris, 1881. P. Manson, Tropical Diseases, Cassell & Co., London, 1900, p. 1. For a full account of the literature, see the monograph by W. S. Thayer and J. Hewetson, "The Malarial Fevers of Baltimore," Johns Hopkins Hosp. Rep., vol. v. On recent advances in our knowledge concerning the etiology of malarial fever, see W. S. Thayer, Phila. Med. Jour., 1900, p. 1046, where a full account of the literature is given. T. B. Futcher, "A Critical Summary of Recent Literature concerning the Mosquito as an Agent in the Transmission of Malaria," Am. Jour. Med. Sci., 1899, p. 318. W. S. MacCallum, "On the Hæmatozoön Infection of Birds," Jour. Exper. Med., vol. iii. p. 117. E. L. Opie, "On the Hæmatozoön of Birds," Ibid., p. 79. F. Grohe, "Zur Gesch. d. Melanaemie," Virchow's Archiv, 1861, vol. xx. 306.

Trypanosomiasis.

The first authentic report on the occurrence of trypanosomes in man was made by Dutton in 1902, while in animals their occasional presence had long been known. They have been described in frogs, dogs, rats, ground-hogs, and horses, and certain species appear to be distinctly pathogenic for some domestic animals in tropical regions.

Especially interesting is the observation of Castellani and Bruce that trypanosomiasis occurs in a large percentage of cases of sleeping sickness. Bruce could demonstrate the organism in the blood in 12 of 13 cases, and in the cerebrospinal fluid obtained by lumbar puncture in all of 38 cases. Castellani had previously found the same parasite in the cerebrospinal fluid in 20 of 34 cases. The question whether the disease is caused by trypanosomes is, however, not as yet decided. Manson, who likewise found the organism, nevertheless expresses himself very reservedly.

FIG. 35.

Trypanosoma gambiense in human blood. (DUTTON.)

The *trypanosoma gambiense* (Dutton) is from 8 to 25 μ long, and from 2 to 2.8 μ broad. It is provided with an undulating membrane and a flagellum, which starts from a centrosome or micronucleus, lying in the posterior end of the animal, and projects somewhat beyond the anterior end (Fig. 35). There is an oval nucleus which is centrally located and is made up of chromatin granules.

In the wet preparation the organism exhibits slow spiral movements. It is found free in the blood-plasma, but may also be seen in the interior of leucocytes, which latter manifestly destroy the organisms exactly as the malarial parasites. In dry specimens the trypanosomes can be readily stained with any basic dye; with the Romanowsky stain or one of its modifications it is stained like the malarial organism. Their number in a blood preparation is rarely

large; as a rule not more than from 3 to 8 are found to a cover-slip. During apyrexia they are not seen. Infection in man probably occurs through mosquitoes.

LITERATURE.—Dutton, Thompson-Yates Laboratory Rep., 1902, vol. iv. Part II., p. 455; and Brit. Med. Jour., 1903, vol. i. p. 304. Castellani and Bruce, Ibid., pp. 1218 and 1431; Jour. Trop. Med., 1903, p. 167.

Spotted Fever.

In the so-called spotted fever, which occurs in Montana, Nevada, Oregon, etc., an intracorpuscular amœboid, non-pigmented organism has been described, which is thought to be the cause of the disease. It sometimes has a terminal dark spot and sometimes occurs in pairs, when it is not amœboid. It is termed the *Pyroplasma hominis*. Infection supposedly takes place through ticks belonging to the species Dermaceutor reticulatus.

LITERATURE.—Wilson and Chowning, Jour. Am. Med. Assoc., 1902, vol. xxxix. p. 131. Anderson, J. F., Am. Med., 1903, vol. vi. p. 506.

Filariasis.

According to Manson, the embryos of at least four, and possibly five and even more distinct species of nematodes may be found in the blood of man. These various blood worms Manson designates as the Filaria nocturna, Filaria diurna, Filaria perstans, Filaria demarquaii, Filaria ozzardi (a doubtful species), and a sixth, which may or may not be connected with one of the two last, the Filaria magelhæsi. Two of these at least are of pathological import, viz., the Filaria nocturna and the Filaria perstans.

Filaria Nocturna (Manson): *syn.*, Filaria sanguinis hominis (Lewis).—This filaria is the embryo form of the Filaria Bancrofti (Cobbold), which inhabits the lymphatics and is unquestionably the cause of endemic chyluria, of various forms of lymphatic varix, of tropical elephantiasis arabum, and possibly also of other obscure tropical diseases. The organism in question is widely distributed. It is indigenous in almost all tropical and subtropical countries as far north as Spain in Europe and Charleston in the United States, and as far south as Brisbane in Australia. It is very common in Cochin and in some of the South Sea Islands, where one-third and one-half of the population, respectively, appear to be infested.

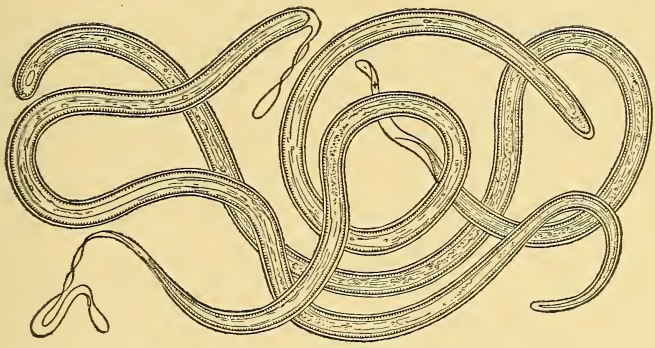
In the following description of both parent and embryo form I quote largely from Manson's account of the parasite in his admirable manual of tropical diseases.

The parent filariæ are hair-like, transparent worms measuring from 7.5 to 10 cm. in length. The sexes live together, often inextricably coiled about each other. Sometimes they are enclosed, coiled several in a bunch, and tightly packed in little cyst-like dilatations of the

distal lymphatics; sometimes they lie more loosely in lymphatic varices; sometimes they inhabit the large lymphatic trunks between the glands, the glands themselves, and probably not infrequently the thoracic duct. The female is the larger; there are two uterine tubes which occupy the greater part of the body, and which are filled with ova in various stages of development. The vagina opens near the mouth; the anus just in advance of the tip of the tail. The cuticle is smooth and without markings. In both sexes the mouth-end tapers slightly; it is clubbed and simple. The male is characterized by its marked disposition to curve. The cloaca gives exit to two slender unequal spicules.

In the wet preparations the *Filaria nocturna* appears as a transparent colorless little worm, which wriggles about most actively, constantly agitating and displacing the corpuscles in its vicinity. It will be noticed, however, that the animal does not propel itself through the drop of blood, but remains stationary. At first the

FIG. 36.



Filaria sanguinis hominis, showing sheath. (After LEWIS.)

movements are so active that it is impossible to make out any anatomical details; after a number of hours, however, the movements become more sluggish, and it is then possible to study the worm with more ease. It measures about 0.31 mm. in length by 0.007–0.008 mm. in width. With the higher power it will be seen that the entire worm is enclosed in a delicate envelope, in which it moves backward and forward, the sheath being much larger than the worm (Fig. 36). It is owing to the presence of this sheath that active locomotion on the part of the worm is not possible. About the posterior part of the middle third of the parasite there is an irregular aggregation of granular matter, which represents a viscus of some sort. With a high power one can further make out a delicate transverse striation in the musculocutaneous layer throughout the entire length of the animal. In stained specimens two V-shaped light spots can be

made out; one at a point about one-fifth of the entire length of the organism, backward from the head-end; the other very much smaller, a short distance from the tail. The first, Manson designates the "V" spot, the second the tail spot. In stained specimens these two spots are readily made out, as they do not take the color. When the movements of the animal have almost ceased, one can see on careful focussing that the head is constantly being covered and uncovered by a six-lipped or hooked, and very delicate prepuce; and, moreover, one can sometimes see a short fang of extreme tenuity suddenly shot out from the uncovered extreme cephalic end, and as suddenly retracted.

Technique.—The examination should be made late in the evening, after the patient has rested for a number of hours. Drops of blood are then mounted, wet, on slides and ringed with vaseline to prevent the specimen from drying. In such preparations the filariæ keep alive for a week or longer. They should be searched for with a low power—an inch objective is very convenient for the purpose. Attention is directed to their presence by the commotion which they cause among the neighboring blood corpuscles.

To prepare permanent mounts, blood-smears are best made on slides which are then stained with the eosinate in the usual manner. Working with the blood of infected animals, I have thus obtained very good results. The V and tail spots are very well brought out. To show anatomical details, however, staining with eosin and hæmatoxylin after fixing the smears with alcohol or heat, gives the best results; in this manner the sheath is very well shown, as also the structure of the musculocutaneous layer.

The number of worms which may be found in a specimen is very variable. During the daytime they are rarely seen, and if at all, only one or two specimens at most are found. As evening approaches, however, commencing about 5 or 6 o'clock, the filariæ enter the peripheral circulation in increasing numbers. At midnight the maximum number is about reached, with from 300 to 600 to the drop of blood. Later they gradually decrease, and by 8 or 9 A. M. they have again disappeared. This periodicity, however, may be reversed if the patient is made to sleep during the daytime and remains awake at nights. During their absence from the peripheral circulation they may be found in the larger arteries and in the lung.

In non-active cases the number of filariæ even at night is quite small. In one instance of this kind I found only the sheath of a single worm while examining perhaps fifty specimens.

Infection occurs through the females of certain mosquitoes, probably of the genus *Culex*, which have fed on the blood of a filaria-infested individual. The history of the parasite while in the body of the mosquito is in brief the following: after their arrival in

the stomach the young worms shed the sheath and invade the thoracic muscles, where they increase in size (to 1.5 mm.), develop a mouth, an alimentary canal, and a trilobed tail. They then find their way into the abdomen, where, in suitably prepared sections, they may occasionally be seen in the tissues about the stomach, and even among the eggs in the posterior part of the abdomen. The majority now find their way to the base of the proboscis and under appropriate conditions out through the proboscis by a channel which they make for themselves. After introduction into the human body the organism finds its way into the lymphatics, where it attains sexual maturity ; fecundation takes place and the new generation of filariæ enter the blood-current by way of the thoracic duct and the left subclavian vein. The development of the embryo form in the mosquito occupies from sixteen to twenty days.

Whether or not infection can occur in any other way is not known, but not impossible. We could conceive that some of the worms are eliminated with the eggs of the mosquito, and that infection could then take place through contaminated drinking-water.

Filaria Perstans.—This species is of interest as it was thought to be concerned in the causation of the so-called sleeping sickness of west tropical Africa. It has likewise been found in the Buck Indians of British Guiana, among whom the same sickness also occurs.[1] The organism observes no periodicity, but is present in the blood both during the daytime and at night.

The embryo worm is smaller than the filaria nocturna ; it measures about 0.2 mm. in length by 0.004 mm. in breadth. It has no sheath, and its caudal end is truncated and abruptly rounded. There is no hooked cephalic prepuce. Its motion is progressive.

The adult form measures 70–80 mm. in length. The tail in both sexes is incurvated and the chitinous covering at the extreme tip split, as it were, into two minute triangular appendages. They have been found in the connective tissue, at the root of the mesentery, behind the abdominal aorta, and beneath the pericardium.

LITERATURE.—Mosler u. Peiper, Specielle pathol. u. Therap., 1894, vol. vi. p. 219. P. Manson, Allbutt's System of Medicine, vol. ii. I. Guitéras, Med. News, April, 1886. F. P. Henry, Ibid., 1896. E. Opie. Am. Jour. Med. Sci., 1901, vol. cxxii. p. 251. P. Manson, Tropical Diseases, Cassell & Co., London, 1900.

Distomiasis (Bilharziasis).

Bilharzia hæmatobia (Cobbold) : *syn.*, Gynæcophorus (Diesing) ; Distomum hæmatobium (Bilharz) ; Schistosoma hæmatobium (Weinland) ; Distoma capense (Harley) ; Thecosoma (Maguin-Tandon).

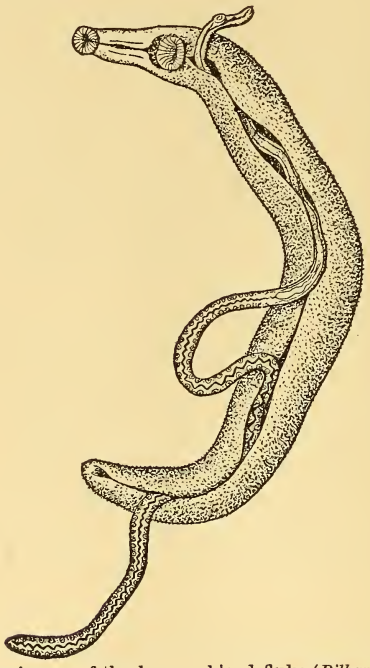
The Bilharzia hæmatobia belongs to the class of trematode

[1] More recent observations tend to throw doubt on this relationship and rather suggest a connection between a species of Trypanosoma and sleeping sickness (see page 191).

platodes. According to Bilharz, the greater portion of the Fellah and Coptic population of Egypt is infested. It is abundant in South Africa, and has also been observed in Mesopotamia, and apparently in Arabia. In the United States a few isolated cases have been seen which were undoubtedly imported. From Europe no endemic cases have been reported. The parasite may give rise to diarrhœa, hæmaturia, and ulceration of the mucous surfaces.

The male is smaller but thicker than the female, measuring from 12 to 15 mm. in length by 1 mm. in breadth. On its abdominal surface a deep groove is found with overlapping edges, which serves for the reception of the female (Fig. 37). It has an oval and a ventral sucker placed close together.

Fig. 37.

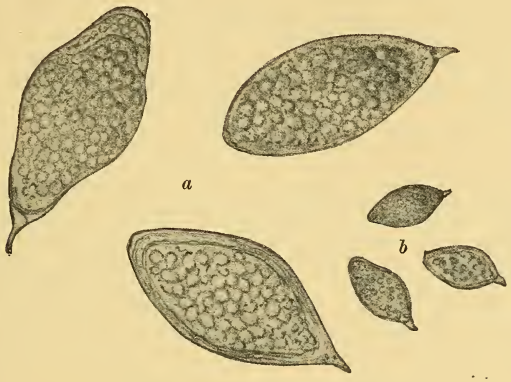


Male and female specimens of the human blood fluke (*Bilharzia hæmatobia*). × 12. (After Looss.)

The adult parasites are found in the blood of the portal vein, in its mesenteric and splenic branches, and in the vesical, uterine, and hemorrhoidal veins; they have also been found in the vena cava and may possibly occur elsewhere in the circulation. The eggs are more often seen. They are oval bodies, measuring 0.16 mm. in length by 0.05 mm. in breadth, and are provided with a distinct, spike-like projection which issues from one extremity or the side (Fig. 38). Infection usually takes place through unfiltered

drinking-water, but may also occur through the skin. Through the portal system the parasite then infests the urogenital system, the

FIG. 38.



Bilharzia eggs from the urine: Group *a* was drawn to scale with B. & L. 1/6 obj., and 1 in. ocular; group *b* represents their appearance with B. & L. 2/3 obj.

anus, and rectum, and may also proliferate abundantly in the intestine, the liver, kidneys, etc. The diagnosis is usually made by examination of the urine, in which the ova will be found.

LITERATURE.—Bilharz, Wien. med. Woch., 1856, vol. vi. p. 49. Meissner, Schmidt's Jahrbuch., 1882, vol. xx. p. 193. Rutimeyer, Verhandl. d. Cong. f. inn. Med., 1822, vol. xi. p. 144.

Anguilluliasis.

In 1995 Teissier reported a case of intermittent fever in which numerous embryos of anguillula were found in the blood. They disappeared after expulsion of the parasites from the intestinal tract, and at the same time the fever ceased. It is a question, however, whether Teissier's parasite was identical with the common form described by Bavay, Normand, Grassi, and others (see page 320). Unlike the embryo developing from the eggs of both parasitic and free living generations, Teissier's form did not present the characteristic double œsophageal enlargement, and he reports, moreover, that in the case of the adult male only one, instead of two, spicules was noted. This view is strengthened by the observation that after inoculation into frogs the worms developed in the intestinal canal and the lungs into giant forms, which may have been *Ascaris nigrovenosa* (*syn.*, Rhabdonema nigrovenosum).

LITERATURE.—Teissier, Compt. rend. de l'acad. des sci., 1895, vol. cxxi. p. 171. Arch. de méd. expér. et d'anat. path., 1895, vol. vii. p. 675; Ibid., 1896, vol. viii. p. 586.

CHAPTER II.

THE SECRETIONS OF THE MOUTH.

SALIVA.

Normal saliva is a mixture of the secretions derived from the submaxillary, sublingual, parotid, and mucous glands of the mouth. It is a colorless, inodorous, tasteless, somewhat stringy and frothy liquid, and serves the purpose of aiding in the acts of mastication, deglutition, and digestion. The quantity secreted in twenty-four hours amounts to about 1500 grammes.

General Characteristics.

Normal saliva has a specific gravity of from 1.002 to 1.009, corresponding to the presence of from 4 to 10 grammes of solids. The reaction of the saliva proper is alkaline, the degree of alkalinity corresponding to from 0.006 to 0.048 per cent. of sodium hydrate. Normally an acid saliva is observed only in newly born infants and in sucklings.

The reaction of the tongue and the mucous membrane lining the mouth is quite commonly acid early in the morning, owing to the production of lactic acid by some of the bacteria which are constantly present in the mouth. This acid, according to Magittot, corrodes the enamel of the teeth, and may ultimately produce dental caries.

Chemistry of the Saliva.

In order to give an idea of the general composition of the saliva the following analyses are appended; the figures correspond to 1000 parts by weight:

Water	995.20	994.20	988.10
Ptyalin[1]	1.34	1.30	1.30
Mucin } Epithelium }	1.62	2.20	2.60
Fatty matter	. .	. .	0.50
Sulphocyanides	0.06	0.04	0.09
Alkaline chlorides	0.84	. .	. .
Disodium phosphate	0.94	2.20	3.40
Magnesium and calcium salts	0.04	. .	. .
Alkaline carbonates	traces.		

[1] These figures are too high, as they refer to the total precipitate obtained with alcohol.

In order to demonstrate the presence of the sulphocyanides, it is usually only necessary to heat a few cubic centimeters of the pure saliva, faintly acidified with hydrochloric acid, with a dilute solution of ferric chloride, when a red color will be seen to develop. If necessary, larger quantities, such as 100 c.c., are evaporated to a small volume; the test is then applied to the concentrated fluid.

Of organic matter, ptyalin, a little albumin mixed with mucin, and about 1 gramme of urea pro liter are found. Of all these substances, the ptyalin is especially interesting from a physiological point of view. It may be isolated in a comparatively pure state according to Gautier's method:

To a large quantity of saliva alcohol (98 per cent.) is added as long as a flocculent precipitate forms. This is collected upon a small filter and dissolved in a little distilled water. The solution thus obtained is treated with several drops of a solution of mercuric chloride, in order to remove albuminous material, which is filtered off. The excess of mercury is removed by means of hydrogen sulphide, when the remaining liquid is evaporated at a temperature of from 35° to 40° C., and taken up with strong alcohol. The insoluble residue is dissolved in a little water, filtered, dialyzed in order to remove inorganic salts, and is finally precipitated with strong alcohol, when the ptyalin will separate out in light flakes. Obtained in this manner, ptyalin is a white amorphous substance, soluble in water, dilute alcohol, and glycerin. In neutral or even slightly alkaline solutions, but not in acid solutions, it rapidly transforms boiled starch into dextrin and sugar at a temperature of from 35° to 40° C. This transformation takes place according to the equations:

(1) $(C_{12}H_{20}O_{10})_{54} + 3H_2O = 3[(C_{12}H_{20}O_{10})_{17}.C_{12}H_{22}O_{11}]$.
Starch. Erythrodextrin.

(2) $3[(C_{12}H_{20}O_{10})_{17}.C_{12}H_{22}O_{11}] + 6H_2O = 9[(C_{12}H_{20}O_{10})_{5}.C_{12}H_{22}O_{11}]$.
Erythrodextrin. Achroödextrin

(3) $9[(C_{12}H_{20}O_{10})_{5}.C_{12}H_{22}O_{11}] + 45H_2O = 54C_{12}H_{22}O_{11} = 54C_{12}H_{22}O_{11}$.
Achroödextrin. Isomaltose. Maltose.

In order to *test for ptyalin*, a few cubic centimeters of saliva are filtered and added to a solution of starch; the mixture is placed in the warm chamber for some time, when it is tested with cupric sulphate or iodine. At first, starch gives a blue color with iodine; after the reaction has proceeded further a red or violet-red color is obtained, indicating the presence of erythrodextrin, while no change in color at all results when achroödextrin only is present. The maltose may be recognized by the fact that it turns the plane of polarization more strongly to the right than glucose; it also reduces Fehling's solution.

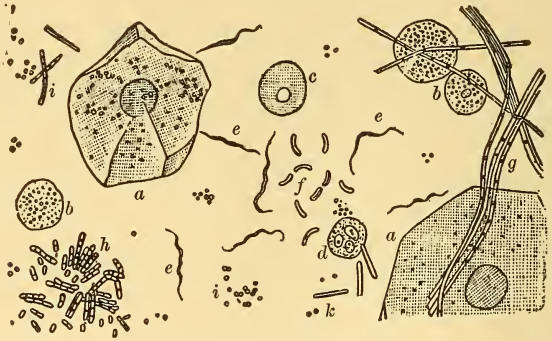
The *test for nitrites*, which may likewise be present in the saliva, is conducted in the following manner: about 10 c.c. of saliva are

treated with a few drops of *Ilasvay's reagent* and heated to a temperature of 80° C., when in the presence of nitrites a red color will develop. The reagent is prepared as follows: 0.5 gramme of sulphanilic acid in 150 c.c. of dilute acetic acid is treated with 0.1 gramme of naphtylamin dissolved in 20 c.c. of boiling water. After standing for some time the supernatant fluid is poured off and the blue sediment dissolved in 150 c.c. of dilute acetic acid. The solution is kept in a *sealed* bottle.

Microscopical Examination of the Saliva.

If normal saliva is allowed to stand, two layers will be seen to form, viz., an upper clear and a lower cloudy layer, which latter contains certain morphological elements. Among these, salivary corpuscles, pavement epithelial cells, and micro-organisms are found (Fig. 39).

Fig. 39.



Buccal secretion. (Eye-piece III., obj. Reichert, $\frac{1}{16}$ homogeneous immersion; Abbe's mirror, open condensers.) *a*, epithelial cells; *b*, salivary corpuscles; *c*, fat-drops; *d*, leucocytes; *e*, Spirochæta buccalis; *f*, comma-bacillus of mouth; *g*, Leptothrix buccalis; *h*, *i*, *k*, various fungi. (v. Jaksch.)

The salivary corpuscles resemble white corpuscles very closely, but differ in their greater size and coarser appearance. The epithelial cells are large, irregular, polygonal cells, provided with well-defined nuclei and nucleoli; they exhibit certain irregularities in size, according to their origin, and belong to the class of pavement or stratified epithelium.

Micro-organisms.[1]—While schizomycetes and moulds are only exceptionally found in the mouth under normal conditions, and are then undoubtedly derived from ingested food, bacteria are always present in large numbers, and it is not surprising that all forms which are found in the air, food, and drink may here be encountered. Some of these, such as the Leptothrix buccalis innominata, Bacillus buccalis maximus, Leptothrix buccalis maxima, Iodococcus vagi-

[1] W. D. Miller, Die Mikroorganismen d. Mundhöhle, 1892.

natus, Spirillum sputigenum, and Spirochæte dentium, are always present. Together with other bacteria, they have been found in carious teeth, in abscesses communicating with the mouth and pharynx, and in exudates on the mucous membranes of these parts. In all probability, however, they are non-pathogenic. To this class also belongs the smegma bacillus, which has been encountered in the saliva, the coating of the tongue, and in the tartar of the teeth of perfectly healthy individuals. In this connection it is interesting to note that, in contradistinction to the bacteria which are only temporarily found in the mouth, the majority of those which are constantly present cannot be cultivated on artificial media.

Important from a practical standpoint is the fact that a number of pathogenic micro-organisms may at times be found under normal conditions. The Diplococcus pneumoniæ, also known as the pneumococcus of Fränkel and Weichselbaum, the Diplococcus lanceolatus, the Micrococcus lanceolatus, the Micrococcus septicæmiæ sputi, and the Micrococcus pneumoniæ cruposæ (Sternberg), has thus been found in a virulent condition in from 15 to 20 per cent. of healthy individuals, and it is even claimed that in a non-virulent state it is *constantly* present in the mouth. Streptococci are likewise frequently observed, but usually possess but little virulence or none at all when obtained from the healthy mouth and tested upon animals. Pyogenic staphylococci may also be found at times, but are less common than the streptococci. Most important is the occasional occurrence of the diphtheria bacillus in the mouths of individuals who have not been exposed to contagion. Welch[1] mentions that virulent organisms were found by Park and Beebe in the healthy throats of eight out of three hundred and thirty persons in New York, who gave no history of direct contact with cases of diphtheria. Two of these eight persons later developed the disease. Non-virulent bacilli were found in twenty-four individuals of the same series, and the pseudodiphtheria bacillus in twenty-seven.

Other pathogenic bacteria which may be found in normal mouths are the Micrococcus tetragenus, the Bacillus pneumoniæ of Friedländer, the Bacillus crassus sputigenus, and the Bacillus coli communis.

It is interesting to note that the secretions of the mouth and throat, as most secretions of the body, possess a certain degree of germicidal power. The Staphylococcus aureus, the Streptococcus pyogenes, the Micrococcus tetragenus, the typhoid bacillus, and the cholera spirillum, when present in moderate numbers, are thus killed by the saliva. The diphtheria bacillus, however, is more resistant, and may survive for twenty-four to forty days. It has been found, as a matter of fact, that the organism may be demonstrated in the throats of some individuals who have passed through

[1] Dennis' System of Surgery: Surgical Bacteriology.

an attack of diphtheria for several weeks after all the clinical symptoms have disappeared. The Diplococcus pneumoniæ is even said to grow well in saliva, although it rapidly loses its virulence. By then cultivating it upon pneumonic sputum, however, the virulence of the organism is restored. The individual bacteria will be considered in detail later on.

Pathological Alterations.

It has been mentioned that about 1500 grammes of saliva are secreted in the twenty-four hours. This quantity is, however, subject to great variation. An increase is thus frequently noted in pregnancy, in various neurotic conditions, in tabes, bulbar paralysis, in inflammatory diseases of the mouth, in dental caries, following the administration of pilocarpin, in poisoning with mercury, acids, and alkalies, etc. The quantity is diminished in all febrile diseases, in diabetes, and often in nephritis. The effect of psychic influences upon the secretion of saliva as well as of other glands is well known, an increase or decrease in the flow being produced under various conditions.

In determining whether or not salivation actually exists, the physician should not only be guided by the statements of his patients, but an actual estimation of the amount secreted within a definite period of time should be made. Hysterical individuals not infrequently complain of "salivation," when a direct estimation will show that the amount is not only not increased, but actually diminished.

An acid reaction of the saliva has been noted in various diseases of the intestinal tract, in febrile diseases, and notably in diabetes (Frerichs). According to Strauss and Cohn, however, an alkaline reaction of the saliva is the rule even under pathological conditions.

Among the qualitative changes may be mentioned an increase in the amount of urea, which has been repeatedly observed, and especially in nephritis.

Urea may be demonstrated as follows: the saliva is extracted with alcohol, the filtrate evaporated, and the residue dissolved in amyl alcohol. This is allowed to evaporate spontaneously, when crystals of urea will separate out, and may then be examined microscopically and chemically (see Urine).

Bile-pigment and sugar have not been found in the saliva.

Of drugs, potassium iodide and potassium bromide rapidly pass into the saliva. Upon this property the indirect examination of the gastric juice for its digestive power—*i. e.*, the presence or absence of free hydrochloric acid—by means of the potassium iodide and fibrin packages of Günzburg, is partly based.

In order to test for potassium iodide, strips of filter-paper moist-

ened with starch solution are immersed in the saliva, which has been acidified with nitric acid; in the presence of potassium iodide the starch-paper turns blue.

SPECIAL DISEASES OF THE MOUTH.

Tuberculosis.—In cases of lupus and the so-called benign form of tuberculosis of the mouth it is rarely possible to demonstrate the presence of tubercle bacilli, even in scrapings taken from the base of the ulcers or in the diseased tissue itself, while in cases of ulcerative stomatitis associated with phthisis in its advanced stages they may be frequently found in large numbers. In some cases, however, their demonstration is by no means easy. In the saliva they are only exceptionally seen.

Actinomycosis.—In cases of actinomycosis it is occasionally possible to demonstrate the presence of the specific organism in or about carious teeth. More commonly, however, the patients are not seen until the primary symptoms of the disease have disappeared, when the typical kernels can no longer be found at the *original* points of entry or have become unrecognizable owing to calcification and retrogressive changes.

Usually the disease has already progressed to the formation of a distinct tumor or abscess, and it may then be necessary to make an exploratory incision, and to examine the scrapings which are brought away. The number of kernels which may be found is at times very small, but a careful examination will probably always lead to their detection if the disease in question is actinomycosis.

Catarrhal Stomatitis.—In this affection the quantity of saliva is increased. Microscopically an increased number of epithelial cells and many leucocytes are noted, their number depending upon the intensity of the morbid process.

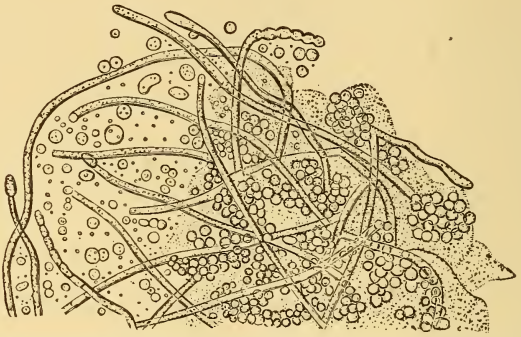
Ulcerative Stomatitis.—In this condition, following mercurial poisoning or scurvy, the same appearance is noted microscopically as in simple stomatitis. In addition there may be necrotic tissue, red blood-corpuscles, and innumerable leucocytes. The reaction of the saliva is intensely alkaline, the color markedly brown, and its odor fetid.

Gonorrhœal Stomatitis.—The number of cases of gonorrhœal stomatitis that have thus far been recorded is small. The disease, however, has received but little attention, and is probably more common than is generally supposed. In the adult it may be contracted through coitus *contra naturam*, while in the newborn the infection is undoubtedly brought about in the same manner as the corresponding disease of the conjunctiva. In suspected cases the exudate which forms upon the gums, the tongue, and the palate should be examined for the presence of gonococci. In adults the

organism has thus far not always been found; in the newborn, however, Rosinski has succeeded in demonstrating its presence in all cases examined.

Thrush.—Oïdium albicans (Fig. 40) is most commonly seen in children, but may also occur in adults, and especially in phthisical individuals, and sometimes lines the entire mouth. If in such cases a bit of the membrane is pulled off and examined microscopically, it will be found to consist of epithelial cells, leucocytes, and granular detritus, with a network of branching, band-like formations, which

Fig. 40.



Oïdium albicans, the vegetable parasite of muguet or thrush. (Reduced from Ch. Robin.)

present distinct segments. The contents of the segments are clear, and usually contain two highly refractive granules—the spores, one of which is situated at each pole. These segments diminish in size toward the end of each band, their contents at the same time becoming slightly granular.

TARTAR.

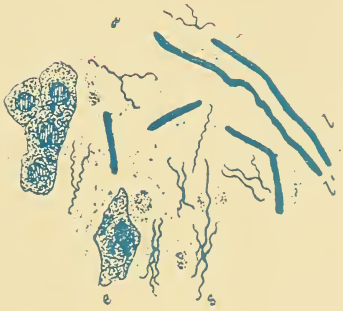
In a bit of tartar scraped from the teeth actively moving spirochætæ are seen, as well as long, usually segmented bacilli, frequently forming bands which are colored bluish red by a solution of iodopotassic iodide. Leptothrix buccalis, shorter bacilli (which are not colored by this reagent), micrococci, and a large number of leucocytes and epithelial cells which have undergone fatty degeneration, are also found. Infusoria have been found by Sternberg, P. Cohnheim, v. Leyden, and others. v. Leyden states that he found infusoria in the tartar in his own person.

COATING OF THE TONGUE.

A brown coating of the tongue is often observed in severe infectious diseases, and consists of remnants of food and incrustated blood. Microscopically, in addition to a large number of epithelial cells, enormous numbers of micro-organisms and a large number of dark,

PLATE XIII.

FIG. 1.



Bacteria of the Mouth. (Cornil Babes.)

FIG. 2.

Leptothrix Buccalis. (v. Jaksch.)

cell-like structures, probably derived from desquamated epithelial cells, are found. The white coating of the tongue contains epithelial cells, many micro-organisms, and a few salivary corpuscles.

COATING OF THE TONSILS.

Pharyngomycosis Leptothrica.

In the pyoid masses derived from the crypts of the tonsils in cases of follicular tonsillitis, and also in persons who have had frequent attacks of tonsillitis, large numbers of lymphocytes of all sizes are seen besides epithelial cells and long, segmented fungi—the Leptothrix buccalis (Plate XIII.)—which are colored bluish red by a solution of iodo-potassic iodide. Ordinary polynuclear neutrophiles are only present in small numbers. At times patches composed of these fungi extend over a considerable area of the tonsils, so that it may be doubtful whether or not the disease is a beginning diphtheria. A microscopical examination will in such cases settle all doubt.

Tonsillitis.

In tonsillitis a large number of bacteria have been isolated from the pseudomembranous deposits. Among the more important which are supposed to bear a causative relation to the disease may be mentioned the various streptococci, staphylococci, less commonly the pneumococcus, the diplococcus of Brison, the bacillus coli communis, the bacillus of Friedländer, the bacillus septicæmiæ sputi, and in a few isolated instances the micrococcus tetragenus. In many cases in which tonsillar deposits are clinically regarded as diphtheritic culture reveals only an abundance of the thrush fungus.

Meyer,[1] in v. Leyden's clinic, succeeded in cultivating a diplostreptococcus from the tonsils of five cases of acute rheumatism with angina, and reports that bouillon cultures of the organism produced characteristic polyarticular arthritis in rabbits. The same organism apparently was also obtained by Allaria[2] in Bozzolo's clinic, and it is interesting to note that his cases resulted from manifest contagion.

In certain cases of ulcerative angina a fusiform diplobacillus and spirochætæ have been found in the exudate by Vincent,[3] Bernheim, and others. The only cases reported from the United States thus far are those of Mayer[4] and Fisher.[5]

[1] F. Meyer, Deutsch. med. Woch., 1901, vol. xxvii. p. 81.
[2] Allaria, Revista critica di clinica Medica, 1901, vol. ii. p. 805.
[3] Vincent, Bull. de la soc. de Hôp., March 11, 1898.
[4] Mayer, Am. Jour. Med. Sci., 1902, vol. cxxiii. p. 187.
[5] Fisher, Ibid., 1903, vol. cxxvi. p. 438.

Glandular Fever.

According to Neumann and Comby, glandular fever generally depends upon infection with a streptococcus. In the case reported by Lande and Froin and by Hirtz[1] bacteriological examination of the throat at the height of the febrile stage revealed the presence of the pneumococcus in a virulent condition.

Diphtheria.

Recognizing the great importance of an early diagnosis in such a malignant disease as diphtheria, an examination for Löffler's bacillus has become just as important to-day as that for the bacillus of tuberculosis.

By means of a sterilized stout platinum loop, a pair of forceps, or a cotton swab, a piece of membrane is scraped from the tonsils, the soft palate, or the pharynx, and is at once transferred to a sterilized test-tube closed with a pledget of cotton. A thin smear is then made either on cover-glasses or slides. If no membrane can be procured, smears are made from a cotton mop. When dry, the specimens are fixed by being passed three or four times through the flame of a Bunsen burner, when they are ready for staining. For this purpose, Löffler's alkaline solution of methylene-blue, which consists of 30 c.c. of a concentrated alcoholic solution of methylene-blue in 100 c.c. of an aqueous solution of potassium hydrate (1 : 10,000) may be advantageously employed, the specimen being stained for from five to ten minutes. It is then rinsed in water, placed on a slide, the excess of water removed with filter-paper, and examined with a $\frac{1}{12}$ oil-immersion lens.

A rapid method of staining, and one which gives even more satisfactory results than that of Löffler, is suggested by Neisser. The organism is grown on blood-serum and examined after from nine to twenty-four hours. The air-dried smears are placed for from one to three seconds in a solution composed of 20 c.c. of an alcoholic solution of methylene-blue (1 to 20 c.c. of 90 per cent. alcohol), 950 c.c. of distilled water, and 30 c.c. of glacial acetic acid. They are then washed in water, stained for from three to five seconds in an 0.2 per cent. hot and filtered aqueous solution of vesuvin, again washed off, dried in the air, and mounted in balsam. The bacilli are brown and have in their interior 2 to 4 blue granules which are usually located near the poles.

A dahlia-methyl-green solution may likewise be employed. This consists of 10 grammes of a 1 per cent. aqueous solution of dahlia-violet and 30 grammes of a 1 per cent. aqueous solution of methyl-green. The specimen is stained for from one to two minutes.

[1] Lande et Froin, Rev. mensuelle des Mal. de l'Enfance, 1901, p. 78.

The following method also may be employed, as suggested by Schauffler. The staining reagent has the following composition :

Filtered solution of Löffler's methylene-blue	10.0 c.c.
Filtered solution of pyronin (0.5 gramme to 10 c.c. of water) . .	1.5 c.c.
Acid alcohol (3 c.c. of 25 per cent. hydrochloric acid to 97 c.c. of absolute alcohol)	0.5 c.c.

Cover-glass specimens are stained for one minute; they are then washed in running water and mounted in balsam as usual. The bacilli are stained blue, the pole bodies a bright ruby red.

Pseudodiphtheritic bacilli are said to take only the blue stain with this method.

If it is desired to employ Gram's method, the specimens are most conveniently stained for three minutes with a freshly prepared concentrated alcoholic solution of gentian-anilin water. This is made by adding anilin oil to 10 c.c. of distilled water, drop by drop, thoroughly shaking after the addition of each drop, until the solution becomes opaque. It is then filtered and treated with 10 c.c. of absolute alcohol and 11 c.c. of a concentrated alcoholic solution of gentian-violet, methyl-violet, or Victoria-blue. The specimen is decolorized in a solution composed of 1 gramme of iodine and 2 grammes of potassium iodide in 300 c.c. of water. After remaining in this solution for five minutes the specimen is differentiated in 95 per cent. alcohol until it ceases to lose color. It is transferred to absolute alcohol, then to oil of cloves or xylol, and mounted in balsam. If desired, one can give the specimen a counterstain, before clearing it, with Bismark-brown. The bacilli retain the blue color.

Cultures should be made, preferably on a mixture of blood-serum and bouillon, as recommended by Löffler. This is composed of 3 parts of blood-serum and 1 part of bouillon, containing 10 per cent. of peptone, 3 per cent. of grape-sugar, and 0.5 per cent. of sodium chloride, the mixture being solidified in the usual manner. Upon this medium Löffler's bacillus grows so much more rapidly than other organisms which are usually present in the secretions of the mouth and throat, that, after from six to eight hours' incubation at 34° to 35° C., they often form the only colonies that attract attention. Smears are then made and stained according to Neisser's method.

In the absence of blood-serum bouillon, alkaline boullion, nutrient gelatin, nutrient agar, glycerin-agar, and potato may be employed. Coagulated egg-albumin, as pointed out by Booker, and milk are also good media. But it is to be noted that the "typical" staining effect with Neisser's method is commonly only obtained if the organism has been grown on ox-blood serum, and if the growth is not older than twenty-four hours.

The colonies are large, round, elevated, and grayish white in

color, with a centre that is more opaque than the slightly irregular periphery. The surface of the colony is at first moist, but after a day or two it assumes a dry appearance.

The bacillus (Fig. 41) is non-motile and varies in size and shape, its average length being from 2.5 to 3 μ, its breadth from 0.5 to 0.8 μ. Its morphological characteristics are so peculiar as to render its identification upon cover-slip preparations and in sections of the diphtheritic membrane an easy matter in most cases.

FIG. 41.

Bacillus diphtheriæ: A, its morphology on glycerin-agar-agar; B, its morphology on Löffler's blood-serum; C, its morphology on acid blood-serum mixture. (ABBOTT.)

Sometimes the organism appears as a straight or slightly curved rod; but especially characteristic are irregular and often bizarre forms, such as rods with one or both ends terminating in little bulbs and rods apparently broken at intervals, in which short, well-defined round, oval, or straight segments can be made out. Very commonly two organisms lie together forming an obtuse angle, or numbers of them may be observed lying side by side.

Some forms stain uniformly, others in an irregular manner; the most typical appearance, as I have already stated, is that of little granules near the poles of the bacillus, which stain blue with Neisser's method, while the body of the organism is colored brown.

Streptococci are also seen, as a rule, and it may be said that the gravity of a case is directly proportionate to the number of streptococci present.

It is important to note that diphtheria bacilli may still be found in the throat for weeks after all clinical symptoms have disappeared.

Patients should hence be isolated until a bacteriological examination has demonstrated the absence of the organism.

LITERATURE.—S. Flexner, "The Bacteriology and Pathology of Diphtheria," Johns Hopkins Hosp. Bull., 1895, p. 39. W. H. Welch, Am. Jour. Med. Sci., 1894. Heubner, Schmidt's Jahrbucher d. gesammten Med., 1892, vol. ccxxxvi. p. 270. Klebs, Arch. f. exper. Path., 1875, vol. iv. p. 207. Löffler, Centralbl. f. Bakt. u. Parasit., 1887, vol. ii. p. 105; and 1890, vol. vii. p. 528. C. Fränkel, "Die Unterscheidung d. echten u. d. falschen Diphtheriebacillen," Berlin. klin. Woch., 1897, p. 1087. W. G. Schauffler, Med. Record, Dec. 6, 1902.

Scarlatina.

According to Baginsky, streptococci are practically constantly found in the pharyngeal secretion.

LITERATURE.—A. Baginsky, Deutsch. med. Woch., Oct. 23, 1902.

CHAPTER III.

THE GASTRIC JUICE AND GASTRIC CONTENTS.

THE SECRETION OF GASTRIC JUICE.

The gastric juice is the result of the glandular activity of the stomach, and is the only secretion of the digestive tract which presents an acid reaction.

As is well known, the mucous membrane of the stomach is covered throughout its entire extent by a single layer of cylindrical epithelium, which dips down in places to line the orifices and larger ducts of the numerous tubular glands with which it is beset. Of these, two kinds are described, viz., the fundus and pyloric glands, so named from the location in which they are principally found. In the secretory portion of a fundus gland two sets of cells can be distinguished. The one kind is small, granular, and polyhedral or columnar, bordering upon the narrow lumen of the tube; these are termed the chief or principal cells (Heidenhain), but are also known as the central or adelomorphous cells. They stain with anilin dyes to only a slight extent. The others, known as parietal, adelomorphous, or oxyntic cells, are variously situated between the adelomorphous cells and the membrana propria; they are most numerous in the necks of the glands. They are larger than the chief cells, oval or angular and finely granular in structure; they possess a strong affinity for the anilin dyes. The pyloric glands, which are found only in the region of the pylorus, on the other hand, are characterized by the greater length of their ducts, which are also lined by the cylindrical epithelium of the mucous membrane proper. The secretory portion of these glands is represented by a single layer of short and finely granular, columnar cells, which closely resemble the chief cells of the fundus glands. In addition to these, a few isolated cells, the cells of Nussbaum, are found, which in structure and in their behavior to anilin dyes resemble the parietal cells.

Upon chemical examination the gastric juice is found to consist essentially of water, free hydrochloric acid, pepsin, rennet (a milk-curdling ferment), mucus, and certain mineral salts.

Of these constituents, the hydrochloric acid is secreted by the parietal cells, pepsin and the milk-curdling ferment by the chief cells of the fundus and the pyloric glands, while the mucus is the product of the cylindrical goblet-cells lining the stomach and the

wider portions of its glandular ducts. It should be borne in mind, however, that the ferments mentioned do not exist in the cells as such, but as zymogens, which are transformed into the ferments through the activity of the free hydrochloric acid. According to modern investigations, moreover, the zymogens only are *secreted* by the cells.

Until recently it was supposed that the gastric juice is secreted only upon appropriate stimulation of the nervous mechanism of the stomach, either directly or indirectly, and that the stomach in its quiescent state—*i. e.*, when not digesting—is empty. The researches of Schreiber and Martius, however, have rendered the correctness of this view doubtful, as they were able to obtain quantities of gastric juice, varying from 1 to 60 c.c., from the non-digesting stomach of every normal person examined. I have likewise never failed to obtain a few cubic centimeters under the same conditions.

TEST-MEALS.

Although the secretion of gastric juice takes place continuously, the amount that can usually be obtained from the non-digesting organ is not sufficient for analytical purposes. It is, therefore, necessary to stimulate the glandular apparatus of the stomach to increased activity. This may be accomplished with thermic, chemical, electrical, and digestive stimuli, of which the last named are the most convenient and the most effective, furnishing an idea not only of the secretory, but also of the motor and resorptive activity of the organ. The analytical results will, however, depend to a large extent upon the character of the food ingested, starches and fats exerting but a slight stimulating effect, while proteids cause a copious secretion of gastric juice. The ingestion of fluids at the same time will likewise influence the results obtained, owing to dilution of the gastric juice. The time of the height of digestion, moreover, varies with the kind and quantity of food taken. In order to obtain uniform results it is necessary, therefore, to withdraw the gastric contents at a certain period after the ingestion of a meal of known composition and bulk.

Numerous test-meals have been proposed. The following are the most important:

The Test-breakfast of Ewald and Boas.

This consists of from 35 to 70 grammes of wheat-bread and of 300 to 400 c.c. of water or weak tea, without sugar. It is best to give this meal to the patient early in the morning, when the stomach is empty—*i. e.*, as a breakfast. The gastric contents are obtained one hour later.

The Test-dinner of Riegel.

This consists of a plate of soup (400 c.c.), a beefsteak (200 grammes), a slice or two of wheat-bread (50 grammes), and a glassful of water (200 c.c.). The contents of the stomach are obtained after four hours. The disadvantage of this method lies in the fact that the lumen of the stomach-tube is frequently occluded by large pieces of undigested meat, a source of annoyance which may be guarded against, however, by using finely chopped meat.

The Double Test-meal of Salzer.

For breakfast the patient receives 30 grammes of lean, cold roast, hashed or cut into strips sufficiently small not to obstruct the stomach-tube; 250 c.c. of milk; 60 grammes of rice; and one soft-boiled egg. Exactly four hours later the second meal is taken, consisting of 35 to 70 grammes of stale wheat-bread and 300 to 400 c.c. of water. The gastric contents are withdrawn one hour later. In this manner the gastric juice is not only obtained at the height of digestion, but an idea may at the same time be formed of the motor power of the stomach. Under normal conditions the organ should contain no remnants of the first meal at the time of examination.

The Test-breakfast of Boas.

This consists of a plateful of oatmeal-soup, prepared by boiling down to 500 c.c. one liter of water to which one tablespoonful of rolled oats has been added. A little salt may be used if desired, but nothing more. The contents of the stomach are obtained one hour later. This test-meal was devised by Boas in order to guard against the introduction from without of lactic acid, which is present in all kinds of bread. The meal is employed in cases of suspected cancer of the stomach in which a quantitative estimation of lactic acid is to be made, the stomach being washed out completely the night before.

Still other test-meals have been suggested, but they possess no material advantage over those described.

THE STOMACH-TUBE.

The stomach-tubes in general use are essentially large Nélaton catheters. They should measure at least from 72 to 75 cm. in length, and be provided with three fenestra, of which one is placed at the end of the tube and two laterally, as near the end as possible. For the purpose of washing out the stomach the tube is connected with a glass funnel by means of ordinary rubber tubing, which can be detached from the stomach-tube proper. There is no advantage in rubber funnels or in having a continuous tube.

It is important that the tubes should be thoroughly cleansed in hot water as soon after use as possible. The advice of Boas, moreover, to have special, marked tubes for tubercular, syphilitic, and carcinomatous patients, should be borne in mind. Patients in whom lavage is to be practised for any length of time should provide their own instruments.

Contraindications to the Use of the Tube.

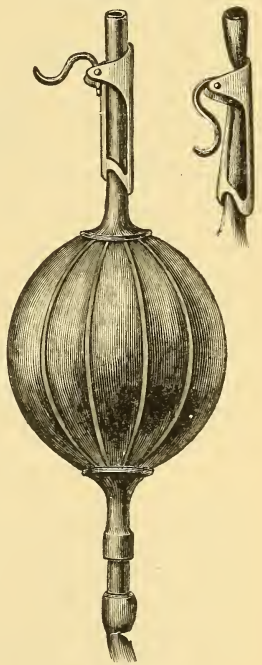
Of direct contraindications to the use of the tube, there should be mentioned the existence of the various forms of valvular disease when in a state of imperfect compensation, angina pectoris, arteriosclerosis of high degree, aneurism of the large arteries, recent hemorrhages from whatever cause, marked emphysema with intense bronchitis, acute febrile diseases, etc.

Introduction of the Tube.

The technique of the introduction of the tube should be as simple as possible; the exhibition of complicated bottle arrangements for the purpose of obtaining the gastric juice only adds to the excitement of a nervous patient, and should be avoided. The patient's clothing and floor of the room should be protected from being soiled by material that may be vomited along the sides of the tube, the dribbling of saliva, etc. For this purpose, Türck's rubber bib with pouch may be advantageously employed. "It is so arranged as to form a pouch in front, to catch the saliva or stomach contents that may be thrown off from the mouth or stomach. A detachable tube passes from the bottom of the pouch and is conducted into a basin or any suitable vessel."[1]

Cocainization of the pharynx is not necessary, but may be resorted to in hyperæsthetic individuals, a 10 per cent. solution being employed.

Fig. 42.



Boas' bulbed tube.

The tube, held like a pen, is passed to the posterior wall of the pharynx, the patient bending his head *forward, and not backward,* as is usually advised. The patient is then told to swallow, but this is not necessary. The tube is pushed on until resistance is felt when it meets with the floor of the stomach. The procedure does not occupy ten seconds. At the least sign of cyanosis or of marked

[1] Manufactured by G. Tiemann & Co., New York.

pallor the tube should be withdrawn at once, and the patient observed for a day or two before a second attempt is made.

If the gastric juice does not flow at once, the patient is instructed to bear down with his abdominal muscles, and, if this is insufficient, to cough a little. Repeated attempts of this kind will usually bring about the desired result, unless the tube has not been introduced far enough or too far; in the latter case it will double upon itself, so that its end rises above the level of the liquid. Pressing upon the abdomen with the hands is of no effect (Method of Expression).

Aspiration must at times be employed. For this purpose, Boas' bulbed tube (Fig. 42) is convenient. The manner in which it is used is the following: the proximal end of the tube, after having been introduced into the stomach, is compressed and the bulb squeezed, when the distal end is clamped and the bulb allowed to expand. When this is repeated several times a partial vacuum is

FIG. 43.

Arrangement of bottle for aspiration of the gastric contents.

produced in the tube, which usually causes a flow of gastric juice. In the absence of such an instrument the stomach-tube may be connected with a bottle, in which a partial vacuum has been established by aspiration (Fig. 43). Unless the patient is accustomed to the introduction of the tube, however, these more complicated procedures should be avoided as much as possible (Method of Aspiration).

I have found that in cases in which gastric juice cannot be obtained by expression the flow may often be started by suction with the mouth, and I regard this method as preferable to the one just described. With due precautions, viz., holding the tube between the fingers near the mouth of the patient, so as to be informed at once, by the sense of touch, when the stomach contents have reached this point, unpleasant results will be obviated. If only a very small amount of gastric juice is present in the stomach—*i. e.*, when a defi-

nite flow cannot be established—it is best to suck lightly with the mouth, to compress the tube firmly, to remove it as rapidly as possible, and empty it into a little dish. A few drops, sufficient to test for free hydrochloric acid, can thus always be obtained, even from the non-digesting organ.

Einhorn's bucket-method is of little value, as the amount of gastric juice which can thus be obtained is insufficient for analytical purposes. It may be employed, however, in patients who are particularly nervous, and who object to the use of the tube, and possibly also when its use is contraindicated. The test for hydrochloric acid can be made, but the information thereby obtained is in itself of comparatively little value.

In order to *wash out the stomach*, the funnel-tube is attached, the funnel filled with lukewarm water or any desired medicated solution, elevated above the head of the patient, and the water allowed to flow. From 500 to 1000 c.c. may be introduced at one time. By suddenly depressing and inverting the funnel over a suitable vessel before all water has left the funnel a siphon arrangement is established and the stomach emptied. It is well to measure the returning water as well as the amount introduced. Should the flow diminish or cease before all the water has been removed, the end of the tube probably stands above the level of the liquid, and the flow can be started again by pushing the tube on further or by withdrawing it a little, as the case may be.

Washing out the stomach soon after the ingestion of a full meal is always very tedious and annoying, if not an impossible procedure, as the fenestra readily become obstructed. Should this occur, the funnel, filled with water, is elevated as high as possible, with a view to overcome the obstruction by hydrostatic pressure; or, if this proves insufficient, the funnel-tube is detached and the obstruction dislodged by means of air, for which purpose a Politzer bag or the bulb of a Boas tube is very convenient.

GENERAL CHARACTERISTICS OF THE GASTRIC JUICE.

Pure gastric juice is an almost clear, faintly yellowish fluid, of a sour taste and a peculiar, characteristic odor. Its specific gravity varies between 1.002 and 1.003, corresponding to the presence of but 0.5 per cent. of solids. Its reaction, owing to the presence of hydrochloric acid, is acid.

Amount.

Very little is known of the total quantity of gastric juice that is secreted in the twenty-four hours. The figure given by Beaumont,[1] viz., 180 grammes pro die, based upon observations made upon the often-quoted Canadian hunter, Alexis St. Martin, is undoubtedly too

[1] Beaumont, Experiments and Observations on the Gastric Juice, Boston, 1834.

low. The amount given by Bidder and Schmidt,[1] viz., that corresponding to about one-tenth of the body-weight, is probably more nearly correct.[2] It may be stated *a priori*, however, that the quantity secreted varies within wide limits, being influenced by numerous factors, and notably by the degree of the appetite and the amount and character of the food taken, especially that of the proteids. The age and sex of the individual, the time of day (notably in its relation to the ingestion of food), the emotions, etc., all influence the glandular activity of the stomach.

From the non-digesting organ, as has been pointed out, from 1 to 60 c.c. of gastric juice may be obtained at one time. The amount which can be procured during the process of digestion, on the other hand, varies with the amount of liquid ingested, the time of expression, the size and motor power of the stomach, and the degree of transudation; the process of resorption probably does not play any part, as it has been ascertained that very little water, if any, is absorbed in the stomach.

According to Boas, from 20 to 50 c.c. of filtrate can normally be obtained exactly one hour after the ingestion of Ewald's test-breakfast.[3]

Abnormally large quantities of gastric juice are practically found only in cases of so-called *hypersecretion*, the "Magensaftfluss" of the Germans, which may occur periodically or continuously. Formerly the presence of appreciable quantities of gastric juice in the non-digesting organ was regarded as conclusive evidence of the existence of this condition, but in the light of Schreiber's researches this position can no longer be maintained. The diagnosis should, hence, only be made when in conjunction with the clinical symptoms of hypersecretion from 100 to 1000 c.c. of pure *gastric juice* can be obtained from the non-digesting organ. To this end, the stomach should be emptied completely by the tube, before retiring, and an examination made on the following morning, no food or liquids being allowed in the meantime.

In various pathological conditions abnormally large quantities of liquid may be obtained, which cannot be regarded as gastric juice, however. Attention will be drawn to these conditions at another place.

CHEMICAL EXAMINATION OF THE GASTRIC JUICE.

Chemical Composition of the Gastric Juice.

As has been briefly shown above, gastric juice consists of water, free hydrochloric acid, certain ferments and their zymogens, and mineral salts. Analyses giving the exact chemical composition of pure, uncontaminated gastric juice in man are wanting, owing to the difficulty

[1] Bidder u. Schmidt, Verdauungssäfte u. d. Stoffwechsel, 1852.

[2] Grünewald's figure—*i. e.*, 1580 grammes—I likewise regard as too low. According to my experience, the daily secretion appears to vary between 2000 and 3000 c.c.

[3] Riegel, Die Erkrankungen des Magens, Part I. p. 88.

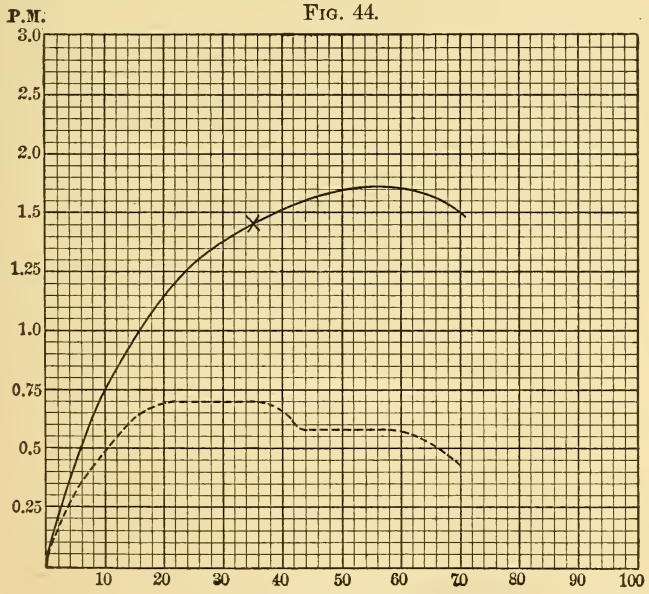
of excluding the saliva. In patients the subjects of gastric fistula analytical studies have, however, been made, and from the table below, taken from Schmidt, an idea may be formed of the various amounts of solid constituents contained in 1000 parts of gastric juice, uncontaminated by food or the products of digestion, but not free from saliva:

Water	994.40
Solids	5.60
Organic material	3.19
Sodium chloride	1.46
Calcium chloride	0.06
Potassium chloride	0.55
Ammonium chloride	. .
Hydrochloric acid	0.20
Calcium phosphate, Magnesium phosphate, Iron phosphate	0.12

The Acidity of the Gastric Juice is Referable to the Presence of Free Hydrochloric Acid.

It has been conclusively demonstrated by Schmidt that the acidity of the gastric juice is due to the presence of free hydrochloric acid.

FIG. 44.



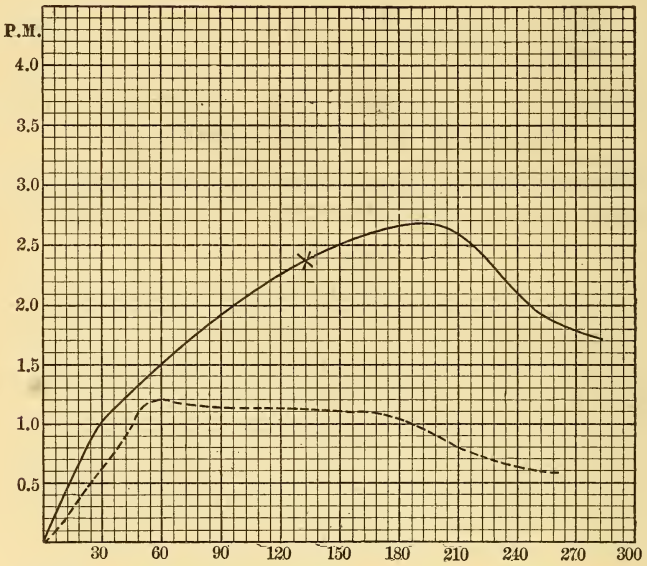
Illustrating the curve of acidity after Ewald's test-breakfast. (ROSENHEIM.)
——Hydrochloric acid. ----Lactic acid. × Beginning of the stage of free hydrochloric acid. P. M. Pro mille. The numbers upon the abscissa indicate the minutes.

After accurately determining the amount of chlorine and all basic substances present, it was found that after the latter had been satu-

rated a quantity of hydrochloric acid still remained, which in the dog varied between 0.25 and 0.42 per cent., with an average of 0.33 per cent. The amount of free acid was also determined by titration and the same results reached as by gravimetric analysis.

While the acidity of pure gastric juice—*i. e.*, gastric juice not contaminated with saliva or food in various stages of digestion—is thus solely due to the presence of free hydrochloric acid, other factors enter into consideration in the examination of the gastric contents during the process of digestion. Acid salts and varying amounts of lactic acid derived from the carbohydrates ingested are

FIG. 45.



Illustrating the curve of acidity after Riegel's test-meal. (ROSENHEIM.)
——Hydrochloric acid. ---- Lactic acid. × Beginning of the stage of free hydrochloric acid.

then also found. At the beginning of digestion the acidity, according to Ewald, is due to a certain extent to the presence of lactic acid.[1] Hydrochloric acid, it is true, is present at the same time, but is held in combination by albuminous material. Later on, when the albuminous affinities have become saturated, it appears as such, with the result that the formation of lactic acid progressively diminishes, owing to the inhibitory action on the part of the hydrochloric acid upon the lactic-acid-producing organisms.[2]

[1] Ewald, Klin. d. Verdauungskrankheiten, 1890, vol. i. Ewald u. Boas, Beitr. z. Physiol. u. Path. d. Verdauung. Virchow's Archiv, 1885, vol. ci. p. 355, and 1886, vol. civ. p. 271. See Lactic Acid, p. 183.

[2] H. Strauss u. F. Bialocour, "Ueber d. Abhängigkeit d. Milchsäuregährung v. HCl—Gehalt d. Magensaftes," Zeit. f. klin. Med., vol. xxviii. p. 567.

The varying degrees of acidity at different periods of digestion, after such test-meals as those of Ewald and Riegel, and the amount of the two acids present, may be seen from the accompanying diagrams (Figs. 44 and 45).

Under pathological conditions the amount of free hydrochloric acid, as will be shown, may undergo great variations, diminishing on the one hand to zero, and increasing on the other to 0.5 per cent., or even more. At the same time the amount of lactic acid, which normally is present in very small amounts, and is absent altogether at the height of digestion, may greatly increase. Fatty acids, moreover, which are normally not present in the gastric juice, may then also be observed. It is thus seen that the total acidity of the gastric juice, especially in disease, cannot be regarded as indicating the amount of one single acid, unless the absence of other acids and acid salts is insured.

Method of determining the Total Acidity of the Gastric Contents.

To this end, a known quantity of gastric juice is titrated with a one-tenth normal solution of sodium hydrate, using phenolphthalein as an indicator, when the number of cubic centimeters of the one-tenth normal solution employed, multiplied by the equivalent of 1 c.c. of this solution in terms of hydrochloric acid, will indicate the amount of acid present, from which the percentage-acidity is readily calculated.

A normal solution of sodium hydrate is one containing the equivalent of its molecular weight in grammes—*i. e.*, 40 grammes—in 1000 c.c. of distilled water; a decinormal solution will, therefore, contain 4 grammes in the same volume of water. This quantity is dissolved in less than 1000 c.c. and the solution brought to the proper strength by titrating it with a solution of oxalic acid of known strength.

From the equation

$$2NaOH + C_2H_2O_4 = C_2Na_2O_4 + 2H_2O,$$

it is seen that two molecules of NaOH (molecular weight 40) combine with one molecule of $C_2H_2O_4 + 2H_2O$ (molecular weight 126), or 4 parts by weight of the former with 6.3 of the latter. One-tenth gramme of oxalic acid would hence require 15.873 c.c. of the one-tenth normal solution of NaOH for its neutralization, as is apparent from the equation

$$6.3 : 1000 :: 0.1 : x;\ 6.3x = 100, \text{ and } x = \frac{100}{6.3} = 15.673.$$

One-tenth gramme of pure crystallized oxalic acid is dissolved in distilled water, and the solution titrated with the one-tenth normal solution of sodium hydrate, which is to be corrected, using two or three drops of a 1 per cent. alcoholic solution of phenolphthalein as an indicator, until the rose color of the solution has entirely disappeared; 15.9 c.c. should bring about this result. As the NaOH solution, however, has been purposely made too strong, less will be required. The amount of water that must then be added in order to bring the solution to its proper strength is determined by the formula $C = \frac{Nd}{n}$, in which C represents the number of cubic centimeters of water which must be added to the remaining solution, N the total number of cubic centimeters remaining after one titration, n the number of cubic centimeters consumed in one titration, and d the difference between the number of cubic centimeters theoretically required and that actually used in one titration. The solution having thus been properly diluted, the correctness of its strength is again tested and a further correction made, if necessary, until absolute accuracy has been attained.

1000 c.c. of the one-tenth normal solution containing 4 grammes of NaOH are equivalent to 3.65 grammes of HCl, as is seen from the equation

$$\underset{40}{NaOH} + \underset{36.5}{HCl} = NaCl + H_2O$$

1000	c.c.	of	the	$\frac{1}{10}$	normal	solution	represent	3.65	grammes of HCl
100	"	"	"	"	"	"	"	0.365	gramme " "
10	"	"	"	"	"	"	"	0.0365	" " "
1	"	"	"	"	"	"	represents	0.00365	" " "

Application to the Gastric Juice.—Five or 10 c.c. of the filtered gastric juice are titrated with the one-tenth normal solution of sodium hydrate, using two or three drops of a 1 per cent. alcoholic solution of phenolphthalein, as an indicator, until the rose color which appears after the addition of every drop of the sodium hydrate solution no longer disappears on stirring or becomes deeper after the addition of a further drop. The number of cubic centimeters of the one-tenth normal solution employed multiplied by 0.00365 will then indicate the acidity of the 5 or 10 c.c. of gastric juice in terms of HCl, from which the percentage-acidity is calculated.

Example.—Ten c.c. of gastric juice required the addition of 6.5 c.c. of the one-tenth normal solution; 6.5×0.00365 (*i. e.*, 0.0237) would hence indicate the acidity of the 10 c.c. of gastric juice in terms of HCl, and $0.0237 \times 10 = 0.237$, the percentage-acidity.

As these figures express the amount of HCl in pure gastric juice obtained only from normal individuals, it has been found more convenient for clinical purposes merely to indicate the degree of acidity by the number of cubic centimeters of the one-tenth normal solution

employed. In the above example, in which 6.5 c.c. were used, the percentage acidity would thus be indicated by the figure 65—*i. e.*, the number of cubic centimeters of the one-tenth solution necessary to neutralize 100 c.c. of gastric juice.

Under normal conditions figures varying from 40 to 60 are usually obtained one hour after the ingestion of Ewald's test-breakfast, while in pathological conditions greater variations are observed. In acute and chronic inflammatory conditions of the stomach, as well as in some of the neuroses, the acidity of the gastric contents is below normal. Higher figures are met with in cases of ulcer, in some cases of dilatation, and are especially frequent in some of the neuroses, in which a degree of acidity corresponding to 90 or even more is not infrequently observed. Increased acidity, usually associated with hypersecretion of gastric juice, is met with in the so-called *hypersecretio acida et continua* of Reichmann.[1]

It has been pointed out that the reaction of normal gastric juice is always acid, owing to the presence of free hydrochloric acid, and the same may be said to hold good for the gastric contents in general, obtained from a normal individual. Pathologically an acid reaction is also the rule, as in those cases in which hydrochloric acid is absent fatty acids and lactic acid usually make their appearance. It is, therefore, not surprising that an alkaline, neutral, or amphoteric reaction is but rarely, or at least not commonly, observed in the gastric contents artificially obtained, and practically seen only in the so-called mucous form of chronic gastritis, or in those rare cases of anadeny, in which a complete destruction of the gastric glands has taken place. In vomited material, on the other hand, such observations are common, owing to the presence of large amounts of saliva. The vomited material in cases of so-called *vomitus matutinus*, which is usually referable to a chronic catarrhal condition of the pharynx, generally presents an alkaline reaction, owing to the fact that the fluid brought up is largely unchanged saliva.

Source of the Hydrochloric Acid.

That the hydrochloric acid is not directly derived from the chlorides ingested is shown by the fact that it is secreted by starving animals. The same point is also proved by the observations of Schreiber, which go to show that the secretion of the acid is continuous, not to mention the well-known fact that even after the ingestion of material free from chlorine an acid gastric juice is secreted. It is apparent, then, that the chlorides of the blood must furnish the necessary chlorine, and as the pyloric glands, which con-

[1] Reichmann, Berlin. klin. Woch., 1882, vol. xix. p. 606; 1884, vol. xxi. p. 768; 1887, vol. xxiv. p. 12.

tain no parietal cells, furnish an alkaline, and the fundus glands, which do contain parietal cells, an acid secretion, it is thought that these parietal cells are in some manner concerned in the production of the hydrochloric acid. The exact manner in which this takes place has not been definitely ascertained, but it is not improbable that the acid results from a "Masseneinwirkung" on the part of the carbonic acid, which is present in large quantities in the blood as such, upon the sodium chloride, and that owing to a specific action on the part of the parietal cells the hydrochloric acid is secreted into the ducts of the glands of the stomach, while the sodium carbonate which is formed at the same time returns to the blood.

Two factors are thus necessary in order that a normal amount of hydrochloric acid should be secreted—*i. e.*, a normal condition of the blood and a normal condition of the cells. Whenever the integrity of either of these factors becomes impaired, it is clear that an abnormal secretion of hydrochloric acid or none at all will result. The nervous system, furthermore, must be taken into consideration as a third factor, as normal innervation is the *sine qua non* for the normal activity of any organ. The secretion of the acid is impaired whenever the nutrition of the cells of the stomach suffers, whether this be the result of inflammatory lesions, new growths, or hyperæmic conditions of the stomach, the effect of renal, hepatic, or pulmonary diseases, etc., or in consequence of central or peripheral nerve influences.

In the *secondary dyspepsias,* then, the result of renal, hepatic, cardiac, or hæmic diseases, etc., an examination of the gastric juice for free hydrochloric acid is of comparatively little value from a diagnostic standpoint, although it may suggest valuable points for the dietetic treatment of such patients.

Significance of Free Hydrochloric Acid.

Formerly it was believed that the principal function of the stomach was a digestive one, and that in the stomach, owing to the action of hydrochloric acid and pepsin, albumins were to a large extent transformed into peptones and albumoses. As pepsin is active only in the presence of a free acid, it was thought, moreover, that the power of the hydrochloric acid to render pepsin physiologically active constituted its entire field of usefulness.

It had been noted over one hundred years ago, however, by the Abbé Spallanzani, that pieces of meat immersed in gastric juice resist the process of putrefaction for days. When it was shown, later on, that the free mineral acids are powerful antiseptics, and that the stomach secretes an amount of free hydrochloric acid sufficient to prevent the development of most of the putrefactive

organisms, the time had come to doubt the correctness of the view previously held.

Numerous experiments have been made in order to test the *antiseptic* and *germicidal power* of the gastric juice. Among the more important results achieved the following may be mentioned: the comma bacillus of cholera Asiatica is destroyed by normal acid gastric juice, while infection results when this has previously been neutralized. The same holds good for numerous other pathogenic organisms which are of special interest to the clinician. Among these may be mentioned the various species of streptococcus, Staphylococcus pyogenes aureus, the bacillus of anthrax, etc. Unfortunately, however, not all species of pathogenic organisms are destroyed by the acid of the gastric juice, and the spores, moreover, of some of those that are destroyed are possessed of a considerable degree of resistance. This is especially true of the tubercle bacillus and in many cases of the spores of the anthrax bacillus.

Those bacteria also which cause lactic acid and butyric acid fermentation resist the antifermentative power of the gastric juice to a certain extent, as may be concluded from the fact that they are always present in the intestines. At the beginning of the process of gastric digestion, when the hydrochloric acid secreted is immediately taken up by the albuminous bodies present, traces of lactic acid can usually be demonstrated in the gastric contents if carbohydrates have been ingested. Later on, when free hydrochloric acid appears, lactic acid fermentation ceases. This observation is in accord with the fact that the action of the lactic acid producers is prevented by the presence of 0.7 pro mille of free hydrochloric acid.

From what has been said it may be argued that as the principal function of the stomach consists in the furnishing of an antiseptic and germicidal fluid, under suitable conditions life could go on in the absence of the stomach. That this is possible has been demonstrated by Czerny, who succeeded in removing almost the entire organ from a dog. Five or six years later the same animal was killed in Ludwig's laboratory, and it was found at the autopsy that "near the cardia a small portion of the stomach had remained, surrounding a globular cavity filled with food." This dog then had lived for almost six years practically without a stomach, had gained in weight, and was to all intents and purposes as healthy an animal as one provided with an entire organ. In the human being similar observations have been made on subjects of carcinoma of the stomach. It is thus very probable that the stomach, so far as the process of digestion is concerned, is not necessary for the maintenance of life.

LITERATURE.—Spallanzani, Experiences sur la digestion de l'homme et de différentes espèces d'animaux, Genève, 1784. Bunge, Lehrbuch d. physiol. Chem., 1889, p. 44. Mester, "Ueber Magensaft u. Darmfäulniss," Zeit. f. klin. Med., vol. xxiv. p. 441. Schmitz, "Zur Kenntniss d. Darmfäulniss." Zeit. f. physiol. Chem., vol. xvii. p. 401; "Die Beziehung d. Salzsäure d. Magensaftes z. Darmfäulniss," Ibid., vol. xix.

p. 401. C. E. Simon, "On Indicanuria," Am. Jour. Med. Sci., 1895, vol. cx. p. 48. Czerny, Beiträge z. operativen Chirurgie, Stuttgart, 1878, p. 141. Ludwig u. Ogata, "Ueber d. Verdauung nach d. Ausschaltung d. Magens," Du Bois' Archiv, 1883, p. 89. J. Carvallo u. V. Pachon, "Untersuchungen über d. Verdauung bei einem Hunde ohne Magen," Arch. der Physiol., 1894, p. 106.

The Amount of Free Hydrochloric Acid.

Pure gastric juice, according to Ewald,[1] Szabó,[2] and Boas,[3] contains from 2 to 3 pro mille of free hydrochloric acid.

In the digesting organ such amounts are met with only at the height of digestion, and after all albuminous and basic affinities have been saturated. The time at which free hydrochloric acid can be demonstrated in the gastric contents after the ingestion of a meal will, hence, vary with the character of the food and its amount. When but little work is to be accomplished free hydrochloric acid is found much sooner than otherwise. After Ewald's test-breakfast, for example, it appears after thirty-five minutes; the point of maximum acidity is reached after from fifty to sixty minutes, and corresponds to the presence of 1.7 pro mille. Following Riegel's meal, on the other hand, the free acid appears after one hundred and thirty-five minutes, and reaches its highest point (corresponding to 2.7 pro mille) in from one hundred and eighty to two hundred and ten minutes (Figs. 44 and 45).

Clinically it is necessary to distinguish between euchlorhydria, or the secretion of a normal amount of free hydrochloric acid (0.1 to 0.2 per cent.), hypochlorhydria, or the secretion of a deficient amount (less than 0.1 per cent.), hyperchlorhydria, in which more than 0.2 per cent. is found, and, finally, anachlorhydria, in which no hydrochloric acid at all is secreted.

Euchlorhydria.—Euchlorhydria, when associated with clinical symptoms pointing to gastric derangement, is most commonly observed in nervous dyspepsia. A chronic gastritis can always be excluded in the presence of a normal amount of the free acid, thus constituting a most important point in the differential diagnosis between the two conditions. A normal secretion of free hydrochloric acid is, furthermore, observed in some cases of atony or hypatony of the muscular walls of the stomach.

Hypochlorhydria.—Hypochlorhydria is associated with all those diseases in which the secretory elements have been more or less damaged, as in subacute and chronic gastritis, in some cases of ulcer of the stomach or the duodenum, in incipient carcinoma, dilatation, and atony.

Anachlorhydria.—Not many years ago it was thought that the absence of free hydrochloric acid from the gastric contents was pathognomonic of carcinoma of the stomach. This view was soon abandoned, however, as it was shown that cases of carcinoma occur

[1] Loc. cit. [2] D. Szabó, Zeit. f. physiol. Chem., 1877, vol. i. p. 155.
[3] Loc. cit. See also A. Schüle, Zeit. f. klin. Med., 1896, vols. xxviii. and xxix.

in which hydrochloric acid is not only present, but present in excessive amounts. This is true especially of those cases in which the malignant growth has started upon the base of an old ulcer. It was, furthermore, shown that anachlorhydria exists in almost all cases of advanced chronic gastritis, and is a very common occurrence in neurasthenic and hysterical individuals, constituting the so-called hysterical anacidity.

Hyperchlorhydria.—The existence of hyperchlorhydria is generally indicative of a gastric neurosis, and is thus frequently met with in its simplest form in certain neurasthenic individuals. Associated with a continuous hypersecretion of gastric juice it constitutes the neurosis that has been described under the term *hypersecretio acida et continua*. Hyperchlorhydria is also of frequent occurrence in cases of gastric ulcer, and may even occur in carcinoma, notably in those cases in which, as stated above, the new growth has started from an old ulcer.

Test for Free Acids.

Following a physical examination of the gastric contents, and, if acid, a determination of the total acidity, the next step will be to determine whether or not the acid reaction is referable to the presence of a free acid, of combined acids, or of acid salts.

The Congo-red Test.[1]—Congo-red is a carmin-colored powder, while its solutions are of a peach- or brownish-red color, which changes to azure blue upon the addition of a free acid, but remains unaffected in the presence of an acid salt. Congo-red may be employed in solution or in the form of a test-paper. The latter, however, is less delicate than the solution, and indicates only the presence of 0.01 per cent. of hydrochloric acid, while a positive reaction can still be obtained with the aqueous solution in the presence of 0.0009 per cent. The solution should be moderately dilute. The test-paper is prepared by soaking filter-paper, free from ash, in this solution, drying, and cutting it into suitable strips. In order to test for the presence of a free acid, it is only necessary to immerse a strip of the test-paper in the filtered gastric juice, or to add a drop or two of the solution to a small amount of the juice, when in the presence of a free acid a blue color will develop, which varies from a sky-blue to a deep azure according to the amount present. A negative result will exclude at once the possibility of peptic activity, as pepsin acts only in solutions containing a free acid. If the result of the test is positive, the nature of the free acid must still be ascertained, and it is, therefore, necessary to test for free hydrochloric acid, or in its absence for lactic acid and certain fatty acids.

[1] Riegel, Deutsch. med. Woch., 1886, No. 35; and Boas, Diagnostik u. Therapie d. Magenkrankheiten.

Tests for Free Hydrochloric Acid.

The various reagents which may be employed are given below, and are arranged according to their degree of delicacy, viz.:

1. Dimethyl-amido-azo-benzol	0.02	pro mille
2. Phloroglucin-vanillin	0.05	"
3. Resorcin	0.05	"
4. Tropæolin 00	0.30	"
5. Mohr's reagent	1.00	"

The Dimethyl-amido-azo-benzol Test.[1]—This test is known also as Töpfer's test, and is destined to replace the older phloroglucin-vanillin and resorcin tests in the clinical laboratory. The delicacy of the reagent is such that the natural yellow color of the indicator is changed to a reddish tinge upon the addition of but one drop of a one-tenth normal solution of hydrochloric acid in 5 c.c. of distilled water. Its superior delicacy, as compared with the phloroglucin-vanillin and resorcin tests, is apparent from the fact that 5 c.c. of a 0.5 per cent. solution of egg-albumin, to which six drops of a one-tenth normal solution of hydrochloric acid have been added, still give a positive reaction with dimethyl-amido-azo-benzol, while the phloroglucin-vanillin and resorcin reactions are negative. Organic acids, including lactic acid, yield a red color only when present in amounts exceeding 0.5 per cent.; I have further ascertained that *if albumoses are present, a cherry-red color is not obtained even though lactic acid be present to the extent of 1 per cent.* Loosely combined hydrochloric acid and salts do not produce a red color.

For practical purposes a 0.5 per cent. alcoholic solution is employed. One or two drops of this are added to a small quantity of the gastric contents, which need not be filtered: in the presence of free hydrochloric acid a beautiful cherry-red develops at once which varies in intensity according to the amount of free acid present. In the presence of organic acids an orange color is obtained. In watery solution the color is a greenish yellow and the fluid is distinctly fluorescent.

To extract the stomach contents with ether, before applying the dimethyl test, as has been suggested, will scarcely ever be necessary.

I have personally used Töpfer's test during the past nine years, and am well satisfied with the results. In teaching students it is well to show the color which one obtains with lactic acid in the presence of albumoses; confusion as to whether or not free hydrochloric acid is present will then not occur.

The Phloroglucin-vanillin Test.[2]—The solution employed contains 2 grammes of phloroglucin and 1 gramme of vanillin, dissolved in 30 c.c. of absolute alcohol: a yellow color results, which gradu-

[1] Töpfer, Zeit. f. physiol. Chem., 1894, vol. xix. Hari, Arch. f. Verdauungskrank. vol. ii. pp. 182 and 332.

[2] Günzburg, Centralbl. f. klin. Med., 1887, vol. viii. No. 40.

ally turns a dark golden red, changing to brown when exposed to light. The solution should therefore be kept in a dark-colored bottle. Lenhartz suggests the use of separate solutions of phloroglucin and vanillin, one or two drops of each being employed in the test. Boas recommends a solution of the phloroglucin and vanillin, in the proportions indicated, in 100 grammes of 80 per cent. alcohol, and claims that the reagent is then still more sensitive and more stable. If a few drops of gastric juice, or even of the unfiltered gastric contents, containing 0.05 per cent. or more of free hydrochloric acid, are treated with the same number of drops of the reagent, no change in color results, but upon the application of gentle heat—*boiling and rapid evaporation are to be avoided*—a rose-tint or exceedingly fine rose-colored lines develop, which are characteristic of the presence of the free acid.

For practical purposes it is best to carry on this slow evaporation on a thin porcelain butter-dish, the porcelain cover of a crucible, or in a small evaporating-dish of the same material. The color obtained in the presence of free hydrochloric acid is a rose color in every instance, and varies in intensity with the amount of acid present. A brown, brownish-yellow, or brownish-red color always indicates that excessive heat has been applied or that free hydrochloric acid is absent.

Organic acids do not produce the reaction, nor is it interfered with by their presence, or that of albumins, peptones, or acid salts.

A phloroglucin-vanillin test-paper, prepared by soaking strips of filter-paper, free from ash, in the solution and drying them, may also be employed. If a strip of this is moistened with a drop of gastric juice and gently heated in a porcelain dish, the rose color will develop in the presence of free hydrochloric acid, and does not disappear upon the addition of ether.

The Resorcin Test.[1]—The solution consists of 5 grammes of resublimed resorcin and 3 grammes of cane-sugar, dissolved in 100 grammes of 94 per cent. alcohol. It is equally as delicate as the phloroglucin-vanillin solution and has the advantage of greater stability.

Five or six drops of gastric juice are treated with three to five drops of the reagent and slowly evaporated to dryness over a small flame, when a beautiful rose- or vermilion-red mirror will be obtained, which gradually fades on cooling. If the reagent is employed in the form of a test-paper, a violet color at first develops, which upon the application of heat turns brick red and does not disappear on treatment with ether.

The presence of acid salts, organic acids, albumins, or peptones does not interfere with the reaction.

[1] Boas, Centralbl. f. klin. Med., 1888, vol. ix. No. 45.

The Tropæolin Test.[1]—Tropæolin 00, when employed according to the method suggested by Boas, is a very reliable reagent, indicating the presence of 0.2 to 0.3 per cent. of free hydrochloric acid. Three or four drops of a saturated alcoholic solution of tropæolin 00, which has a brownish-yellow color, are placed in a small porcelain dish or cover, and allowed to spread over the surface. A like amount of gastric juice is then added and likewise allowed to flow over the surface of the dish; upon the application of gentle heat beautiful lilac or blue stripes appear, which are said to be absolutely characteristic of free hydrochloric acid.

A tropæolin test-paper may also be prepared by soaking filter-paper, free from ash, in the alcoholic solution, and then drying and cutting it into strips. A few drops of gastric juice containing free hydrochloric acid produce a more or less pronounced brown color upon this paper, which turns lilac or blue upon the application of gentle heat. Organic acids, when present in large amounts, likewise produce a brown color, but this disappears on heating, and a lilac or blue color does not result.

For ordinary purposes this test is sufficient, and recourse need only be had to the more delicate reagents when a negative or a doubtful result is obtained.

Mohr's Test, as modified by Ewald.[2]—Two c.c. of a 10 per cent. solution of potassium sulphocyanide are treated with 0.5 c.c. of a neutral solution of ferric acetate, and diluted to 10 c.c. with distilled water, a ruby-red solution resulting. Of this, a few drops are placed in a porcelain dish, when a drop or two of the filtered gastric contents are allowed to come into contact with the reagent. In the presence of free hydrochloric acid a light-violet color develops at the point of contact between the two fluids, and turns a deep mahogany-brown upon mixing.

The test is not interfered with by the presence of acid salts or peptones, but is not so sensitive as those already described.

The Benzopurpurin Test.[3]—Benzopurpurin 6B has been highly recommended by v. Jaksch as a very sensitive test for hydrochloric acid. It is best used in the form of a test-paper, prepared by soaking strips of filter-paper, free from mineral ash, in a concentrated watery solution of the reagent and allowing them to dry.

In the presence of more than 0.4 gramme of hydrochloric acid in 100 c.c. of gastric juice the color of the test-paper immediately turns a deep blackish-blue. Should a brownish-black color develop, this is likely due to the presence of organic acids, or, a mixture of these and hydrochloric acid. If the color is caused by or-

[1] Ewald, Klinik d. Verdauungskrank., Berlin, 1888, vol. ii.; and Boas, Deutsch. med. Woch., 1877, vol. xiii. p. 852.
[2] Ewald u. Boas, Virchow's Archiv, vol. ci. p. 325; vol. civ. p. 271.
[3] v. Jaksch, Klinische Diagnostik, 1896, p. 177.

ganic acids only, it will disappear on washing the strip with a little neutral ether, the original color of the test-paper being thus restored; but if due to a mixture of the two, the reaction is less marked, and does not disappear. According to Hellström,[1] 0.39 milligramme of hydrochloric acid, dissolved in 6 c.c. of water, can be recognized by the addition of only 5 milligrammes of benzopurpurin.

Acid salts, peptones, and serum-albumin do not seriously interfere with the reaction.

v. Jaksch claims that the benzopurpurin test-paper is more sensitive than the Congo-red paper.

The Combined Hydrochloric Acid.

It has been stated (see page 217) that the total acidity of the gastric juice can only be referred to hydrochloric acid when organic acids and acid salts are absent. But at the same time the free acid is titrated together with the loosely combined acid. The presence of free hydrochloric acid in normal amounts implies, of course, the existence of peptic activity, and indicates that all albuminous affinities have been saturated. In the absence of free hydrochloric acid, however, it is important to know whether or not hydrochloric acid is secreted at all—*i. e.*, whether peptic digestion is at a standstill or whether an amount is secreted that is sufficient to saturate only certain albuminous affinities without appearing in the free state. In the treatment of the various forms of gastric disease, more especially those associated with an absence of free hydrochloric acid, accurate knowledge in this respect is important. If no hydrochloric acid at all is secreted, the stomach can only be regarded as a storehouse, as it were, and proteids must be ordered in such a form that they may be subjected to the process of pancreatic digestion with as little delay as possible, the nutrition of the body being aided, if necessary, by a suitable administration of predigested food. If, on the other hand, an amount of hydrochloric acid is secreted which is sufficient to saturate the albuminous affinities of an ordinary meal, or at least of moderate amounts of proteids, the dietetic directions need not be so stringent. While in the former case the absence of loosely combined hydrochloric acid usually indicates complete destruction of the glandular elements of the stomach—in other words, an irreparable condition—a fair prognosis may be given when the amount of acid secreted is sufficient for the saturation of the albuminous affinities of an ordinary meal. The following table[2] shows the amount of hydrochloric acid necessary to saturate the affinities of known quantities of various articles of food, the figures given having reference to 100 c.c. or 100 grammes:

[1] Cited by v. Jaksch.

[2] Taken, in part from personal observations, and in part from Ehrlich, Dissert., Erlangen, 1893.

Milk	0.32–0.56 gramme of pure HCl.
Beef (boiled)	1.95–2.0 grammes " "
Mutton (boiled)	1.9 " " "
Veal (boiled)	2.2 " " "
Pork (boiled)	1.5–1.6 " " "
Sweetbread (boiled)	0.9–0.95 gramme " "
Calves' brains (boiled)	0.56–0.65 " " "
Ham (raw)	1.9 grammes " "
Ham (boiled)	1.3–1.8 " " "
Flounder	1.41 " " "
Liver sausage	0.8–0.9 gramme " "
Cervelat sausage	1.1 grammes " "
Mettwurst	1.0 gramme " "
Bologna sausage	1.49 grammes " "
Blood sausage	0.3 gramme " "
Potato (mashed)	0.48 " " "
Rice (milk)	1.22 grammes " "
Corn	0.27 gramme " "
Graham bread	0.3 " " "
Pumpernickel	0.7 " " "
Wheat bread	0.3–0.5 " " "
Rye bread	0.3–0.5 " " "
Swiss cheese	2.6–2.7 grammes " "
Fromage de Brie	1.3 " " "
Edam cheese	1.4 " " "
Roquefort cheese	2.1 " " "
Beer (German)	0.07–0.15 gramme " "

Quantitative Estimation of the Hydrochloric Acid of the Gastric Juice.

Töpfer's Method.[1]—The free and combined hydrochloric acid is most conveniently estimated according to Töpfer's method, which is both simple and sufficiently accurate for clinical purposes.

In this method the total acidity (*a*) of a given amount of gastric juice—*i. e.*, the acidity referable to the presence of free hydrochloric acid, combined hydrochloric acid, acid salts, and any organic acids that may be present—is first determined (lactic acid and the fatty acids, if present, need not be removed), using phenolphthalein as an indicator. This is followed by a determination of the acidity referable to free acids and acid salts in the same amount of gastric juice (*b*), using alizarin (alizarin monosulphonate of sodium) as an indicator. As this does not react with loosely combined hydrochloric acid, the difference between *a* and *b* will indicate the amount of the latter. The free hydrochloric acid (*c*) finally is estimated with dimethyl-amido-azo-benzol as an indicator, the difference between *a* and $b+c$ giving the acidity referable to organic acids and acid salts.

The solutions required are the following:

1. A decinormal solution of sodium hydrate.
2. A 1 per cent. alcoholic solution of phenolphthalein.
3. A 1 per cent. aqueous solution of alizarin.
4. A 0.5 per cent. alcoholic solution of dimethyl-amido-azo-benzol.

Three separate portions of 5 or 10 c.c. of filtered gastric juice are measured into three small beakers or porcelain dishes. To the

[1] Loc. cit.

first portion 1 or 2 drops of phenolphthalein are added, when it is titrated with the one-tenth normal solution of sodium hydrate. It is necessary, however, to titrate to the point of a deep red, and not to the rose hue which first appears. It will be seen that upon the addition of the first few drops of the one-tenth normal solution the red color, which first appears, disappears on stirring. Upon further titration a point is reached when this no longer occurs, and the color of the entire solution suddenly turns to a rose. This, however, is not the end-reaction that is desired. If the titration is continued, it will be observed that a dark-red cloud forms in the light rose-colored solution, which disappears on stirring; finally, a point is reached when an additional drop no longer intensifies the color of the solution. This point is the end-reaction which must be reached.

To the second portion 3 or 4 drops of the alizarin solution are added, when it also is titrated with the one-tenth normal solution of sodium hydrate until a pure violet color is obtained. As practice is required in order to determine this point with accuracy, Töpfer advises to make previously the following simple tests:

1. To 5 c.c. of distilled water add 2 or 3 drops of alizarin solution, when a yellow color will result.

2. To 5 c.c. of a 1 per cent. solution of disodium phosphate add the same number of drops, when a red or slightly violet color will be obtained.

3. Five c.c. of a 1 per cent. solution of sodium carbonate, treated with 2 or 3 drops of the alizarin solution, will strike a pure violet; this is the color to which the titration must be carried.

In the third portion of the gastric juice the free hydrochloric acid is titrated, after the addition of 3 or 4 drops of the dimethyl-amido-azo-benzol, until the last trace of red—in the presence of free hydrochloric acid—has disappeared, and the color has become distinctly greenish yellow. The results are then calculated as in the following example:

Ten c.c. of gastric juice, using phenolphthalein as an indicator, required 10 c.c. of the one-tenth normal solution in order to bring about the end-reaction, while a like amount titrated in the same manner with alizarin required 7 c.c. in order to bring about the same result. The difference between 10 and 7—*i. e.*, 3—would thus indicate the number of cubic centimeters necessary to neutralize the amount of hydrochloric acid in combination with albuminous material. As 1 c.c. of the one-tenth normal solution represents 0.00365 gramme of hydrochloric acid, the amount of acid thus held will be equivalent to $0.00365 \times 3 = 0.01095$ gramme of hydrochloric acid—*i. e.*, 0.1095 per cent.

In the estimation of the free hydrochloric acid, 2.3 c.c. of the one-tenth normal solution were required, using dimethyl-amido-azo-ben-

zol as an indicator; this would correspond to 0.00365×3.2—*i. e.*, 0.1168 per cent. The value of the total acidity in terms of hydrochloric acid is $10 \times 0.00365 = 0.0365$ gramme for every 10 c.c. of gastric juice, or 0.365 per cent. By deducting the amount of the free and combined hydrochloric acid, viz., $0.1095 + 0.1168 = 0.2263$, from this, it is found that the acidity of the gastric juice referable to organic acids and acid salts amounts to 0.1387 per cent., so that the results can be tabulated as follows:

Free hydrochloric acid	0.1168 per cent.
Combined hydrochloric acid	0.1095 "
Organic acids and acid salts	0.1387 "
Total acidity	0.3650 per cent.

If free acid is absent, the deficit can be ascertained by titrating with decinormal hydrochloric acid, using dimethyl as an indicator.

Estimation of Free Hydrochloric Acid (according to Sahli).—25–30 drops of Günzburg's reagent are added to 10 c.c. of gastric juice. The mixture is titrated with a decinormal sodium hydrate solution as usual until a drop of the mixture, warmed on the stirring-rod after each addition of the alkali, shows a red color. A porcelain dish can, of course, also be used, as in the qualitative test.

The Method of Martius and Lüttke (modified).[1]—This method is equally exact, but requires a greater expenditure of time. It is based upon the fact that upon incineration of the gastric juice the free hydrochloric acid and that loosely combined with albuminous material escape, while the chlorine in combination with inorganic bases remains in the mineral ash unless a very intense heat is applied for some time. By subtracting the amount of chlorine present in the latter form from the total amount, the quantity in combination with albuminous material and that occurring as free acid will be found. The total acidity of the gastric juice is then determined, and that referable to the presence of the free and combined hydrochloric acid subtracted, the difference giving the amount of organic acids present. By determining the acidity due to the presence of free hydrochloric acid according to Töpfer's method, and deducting the amount found from that referable to the presence of free and combined hydrochloric acid, the amount of the latter is obtained.

Reagents required:

1. A solution of silver nitrate in nitric acid of such strength that 1 c.c. shall represent 0.00365 gramme of hydrochloric acid.
2. Liquor ferri sulphurati oxydati.
3. A decinormal solution of ammonium sulphocyanide.
4. A one-tenth normal solution of sodium hydrate.
5. A 1 per cent. alcoholic solution of phenolphthalëin.

[1] F. Martius u. L. Lüttke, Die Magensäure des Menschen, Stuttgart, 1892.

6. A 0.5 per cent. alcoholic solution of dimethyl-amido-azo-benzol.

Preparation of the solutions:

1. The silver nitrate solution. As a solution is required of such strength that 1 c.c. shall be equivalent to 0.00365 gramme of hydrochloric acid, the amount of silver nitrate that must be dissolved in 1000 c.c. of water is ascertained in the following manner: since 169.66 (molecular weight) parts by weight of silver nitrate combine with 36.5 parts of hydrochloric acid (molecular weight), the amount of silver nitrate required for each cubic centimeter is found from the equation

$$169.66 : 36.5 : : x : 0.00365 ; \; 36.5\,x = 0.6192590 ; \; x = 0.0169.$$

In 1 c.c. of the silver solution 0.0169 gramme of silver nitrate must thus be present, or 16.9 grammes in the liter. This quantity, or roughly 17 grammes, is weighed off and dissolved in 900 c.c. of a 25 per cent. solution of nitric acid; as the acid must be present in excess, the solution is purposely made too strong. To this solution 50 c.c. of the liquor ferri sulphurati oxydati are added. The solution is then brought to the proper strength by titration of a known number of cubic centimeters of a one-tenth normal solution of hydrochloric acid and correcting as usual.

2. The ammonium sulphocyanide solution. A normal solution of ammonium sulphocyanide contains 75.98 grammes (molecular weight) per liter, and a decinormal solution 7.598 grammes. This quantity, or roughly 8 grammes, is dissolved in about 900 c.c. of water and the solution brought to the proper strength by titrating a known number of cubic centimeters of the silver nitrate solution, when each cubic centimeter should correspond to 1 c.c. of the silver solution—*i. e.*, to 0.00365 gramme of hydrochloric acid. It is corrected as described elsewhere.

Method.—1. To determine the total amount of chlorine present: 10 c.c. of filtered gastric juice—Martius and Lüttke make use of the unfiltered gastric contents—are measured into a small flask bearing a 100 c.c. mark, and treated with an excess of the one-tenth normal solution of silver nitrate. Experience has shown that 20 c.c. are sufficient. The mixture is agitated and allowed to stand for ten minutes. Distilled water is then added to the 100 c.c. mark; the mixture is agitated once more and filtered through a dry filter into a dry beaker. Fifty c.c. of the filtrate are titrated with the one-tenth normal solution of ammonium sulphocyanide until the blood-red color which appears upon the addition of every drop—due to the formation of ferric sulphocyanide—no longer disappears on stirring. By multiplying the number of cubic centimeters of the ammonium sulphocyanide solution used by 2 (the number of cubic centimeters that would have been necessary for the precipitation of the excess of silver in 100 c.c.) and deducting the result from the

number of cubic centimeters of the one-tenth normal solution of silver nitrate employed, viz., 20, the number of cubic centimeters of the latter solution is found which was necessary to precipitate the chlorine in 10 c.c. of the gastric juice. As 1 c.c. of the solution represents 0.0036 gramme of hydrochloric acid, it is only necessary to multiply this figure by the number of cubic centimeters used in precipitation of the chlorine. The resulting value. *T*, expresses the total amount of chlorine present.

As a general rule, it is not necessary to decolorize the gastric juice. If desired, however, 5 to 15 drops of a 5 per cent. solution of potassium permanganate may be added to the 10 c.c. employed, after the mixture has stood for ten minutes.

2. Determination of the amount of chlorine in combination with inorganic bases, *F*. Ten c.c. of the filtered gastric juice are carefully evaporated to dryness in a platinum crucible, on a water-bath or upon a plate of asbestos, in order to avoid sputtering (as the heat applied in the process of incineration is not very intense, a porcelain crucible may also be employed). The residue is then carefully incinerated over an open flame, the process being carried only to the point when the organic ash no longer burns with a luminous flame. Intense heat should be avoided, as the chlorides are volatilized upon the application of red heat. On cooling, the ash is moistened with a few drops of distilled water and mixed with a stirring-rod, when the residue is extracted in separate portions with 100 c.c. of hot distilled water and filtered. This amount is usually sufficient to dissolve all the chlorides present. If any doubt should exist, however, it is only necessary to add a drop of the silver solution to a few drops of the last portion of the filtrate: the formation of a cloud, referable to silver chloride, will necessitate still further washing. The whole filtrate is then treated with 10 c.c. of the one-tenth normal solution of silver nitrate, and the amount consumed in the precipitation of the chlorides determined by titration with the one-tenth normal solution of ammonium sulphocyanide, as described above. The hydrochloric acid present in combination with inorganic bases is thus determined. The difference between the amount of hydrochloric acid in combination with inorganic bases and the total amount of chlorine in terms of hydrochloric acid will then indicate the amounts of the free and of the combined hydrochloric acid, which are termed *L* and *C*, respectively; hence $T - F = L + C$.

3. The total acidity in terms of hydrochloric acid is further determined according to the method given elsewhere (see page 157) and indicated by the letter *A*. The difference between the total acidity and the amount of free and combined hydrochloric acid will represent the amount of organic acids and acid salts, *O*; hence $O = A - (L + C)$.

The free hydrochloric acid finally is determined according to the method of Töpfer. The difference between the value thus found and that expressing the amount of free and combined hydrochloric acid will indicate the amount of the latter; hence $(L + C) - L = C$.

Leo's Method.[1]—This method is based upon the observation that calcium carbonate combines with free and combined hydrochloric acid at ordinary temperatures to form neutral calcium chloride, while the acid phosphates are not affected. It is thus clear that by determining the total acidity of the gastric juice, and deducting from this the acidity referable to acid salts, the amount of the physiologically active hydrochloric acid—*i. e.*, of the free and combined hydrochloric acid—is obtained.

As it has been shown that in the presence of calcium chloride (formed, as indicated above, upon the addition of calcium carbonate), owing to the formation of calcium monophosphate—$CaHPO_4$, twice the quantity of sodium hydrate is taken up, it is necessary to make the first titration also after the addition of an excess of calcium chloride.

Reagents required:

1. A one-tenth normal solution of sodium hydrate.
2. A 1 per cent. alcoholic solution of phenolphthalein.
3. A concentrated solution of calcium chloride.
4. Chemically pure calcium carbonate. The purity of the salt may be tested by stirring a small piece with water; the solution should not color red litmus-paper blue. A solution of the salt in dilute hydrochloric acid should not yield a precipitate when treated with sulphuric acid.

Method.—Organic acids that may be present are first removed by shaking with ether, 50 to 100 c.c. being required for each 10 c.c. of gastric juice. The total acidity of the gastric juice is then determined in 10 c.c. of the filtered liquid after the addition of 5 c.c. of the concentrated solution of calcium chloride, the result being termed A.

The acidity referable to the presence of acid phosphates is determined as follows: 15 c.c. of filtered gastric juice are treated with a pinch of dry and chemically pure calcium carbonate; the mixture is thoroughly stirred, and passed at once through a dry filter. Ten c.c. of the filtrate, from which the carbon dioxide is expelled by means of a current of air, are then treated with 5 c.c. of the calcium chloride solution and titrated as above, the resulting value being termed P. $A - P$ is hence equivalent to $L + C$. The value of C can then be ascertained by determining the acidity referable to free hydrochloric acid according to Töpfer's method, and deducting the value found from $L + C$.

[1] Leo, Centralbl. f. d. med. Wiss., 1889, vol. xxvii. p. 481.

This method is sufficiently accurate for practical purposes, and has the advantage of not requiring the expenditure of much time.

The Ferments of the Gastric Juice and their Zymogens.

Pepsin and Pepsinogen.—According to our present knowledge, the zymogen of pepsin, viz., pepsinogen or propepsin, and not pepsin itself, is secreted by the chief cells of the fundus glands. This view is based upon the observation that an aqueous extract of the mucous membrane of the stomach of a fasting animal recently killed does not lose its digestive power for a considerable length of time when treated with a 1 per cent. solution of sodium carbonate at a temperature of from 38° to 40° C., whereas pepsin itself is thus rapidly destroyed. It is natural then to conclude that the glands of the stomach do not contain pepsin, but some other substance during the process of fasting, which is capable of resisting the action of sodium carbonate, and which can be transformed into pepsin by the addition of hydrochloric acid. This substance has been termed *pepsinogen* or *propepsin*. As a rule, *pepsin* is obtained only from the mucous membrane of the digesting organ, while at other times the physiologically inactive zymogen is found. As the zymogen, moreover, is probably always present together with pepsin in the gastric juice obtained from healthy individuals during the process of digestion, it is not clear whether the transformation of the zymogen into its ferment takes place in the body of the cell or after secretion. There is evidence to show, however, that the latter view is correct.[1]

This is not the place to enter into a detailed consideration of the various properties of pepsin, and it will suffice to say that the activity of the ferment is destroyed by even very dilute solutions of the alkaline carbonates. The same result is reached by exposing a watery solution of pepsin to a temperature of 70° C., while in a dry state a temperature of 100° C. will not destroy its activity; this is shown by the fact that a specimen of pepsin thus treated is, on cooling, still capable of digesting albumins in the presence of hydrochloric acid.

While pepsin is capable of digesting albumins in the presence of other acids, viz., phosphoric, sulphuric, oxalic, acetic, lactic, and salicylic acids, the solutions must be stronger than in the case of hydrochloric acid. With lactic acid, for example, a satisfactory result is reached only with a concentration of from 12 to 18 pro mille, while of hydrochloric acid 2 to 4 pro mille are sufficient. Larger or smaller amounts do not act so promptly.

Very important from a practical standpoint is the fact that but small quantities of pepsin are required to digest large amounts of albumin. Petit[2] thus claims that a pepsin preparation from his

[1] C. E. Simon, Physiological Chemistry, Lea Bros. & Co., 1901.
[2] Petit, "Étude sur les ferments digestifs," Jour. de. Thérap., 1880.

laboratory was capable of dissolving 500,000 times its weight of fibrin in seven hours. This property possessed by pepsin, of doing an amount of work that is widely out of proportion to the amount of ferment present, is common to all ferments, and is dependent upon the fact that the ferment itself undergoes no change during the process.

Figures expressing the exact quantity of pepsin or of its zymogen produced in the twenty-four hours are lacking, and inferences can hence only be drawn as to the physiological activity of the ferment from the rapidity with which given amounts of albuminous material are digested. This, however, depends to a large extent upon the nature and concentration of the free acid present. Under normal conditions 25 c.c. of gastric juice will dissolve 0.05 to 0.06 gramme of serum-albumin in one hour, the same amount of coagulated egg-albumin in three hours, and a like amount of fibrin in one hour and a half.

As abnormalities in the circulation and innervation of the stomach apparently do not influence the production of pepsin, or rather of its zymogen, a diminution in the degree of peptic activity, or its total absence, may be referred directly to disease of the stomach itself, viz., its glandular apparatus. The determination of the presence or absence and relative amount of pepsin in the gastric juice, hence, furnishes more useful information than the recognition of the presence or absence of free hydrochloric acid.

As pepsin is formed from pepsinogen through the agency of a free acid, its presence, in the absence of organic acids in notable quantities, indicates at once the presence of hydrochloric acid. It may be said, *vice versa*, that if free hydrochloric acid is present in the gastric juice, and the latter digests albumins, pepsin also will be found. Should the zymogen alone be present, digestion will take place only upon the addition of an acid, while an absence of digestion upon the addition of hydrochloric acid indicates the absence of both pepsin and its zymogen. At times, though rarely, a "gastric juice" is met with which is capable of digesting albumin in the absence of hydrochloric acid, owing to the presence of pancreatic juice—a point which is important, both from a diagnostic and a prognostic point of view.

In the differential diagnosis of a chronic gastritis and a neurosis, or a dyspeptic condition referable to hyperæmia of the gastric mucous membrane, the demonstration of the presence of the zymogen in the absence of hydrochloric acid may, at times, be very important, bearing in mind the fact that circulatory and nervous disturbances apparently do not influence the production of pepsinogen. An entire absence of the latter would, of course, warrant the diagnosis of complete anadeny of the stomach.

Tests for Pepsin and Pepsinogen.—Test for the Enzyme.—If the presence of free hydrochloric acid has previously been ascertained, 25 c.c. of filtered gastric juice are set aside and kept at a temperature of from 37° to 40° C., a bit of coagulated egg-albumin, fibrin, or serum-albumin being added. In order to permit of a comparison of results, the same amounts should always be taken; 0.05 to 0.06 gramme of egg-albumin, as has been shown, ought, under physiological conditions, to be digested in three hours.

Test for the Zymogen.—Should hydrochloric acid be absent, the test is made in the same manner, after the addition of from 3 to 5 drops of the officinal solution of hydrochloric acid to 25 c.c. of the filtrate. Under such conditions usually pepsinogen alone is found.

Quantitative Estimation.—Of Pepsin.—Accurate methods for the quantitative estimation of pepsin are unknown, and relative values only can be obtained.

Hammerschlag's Method.[1]—Three Esbach's tubes (albuminimeters) are employed. Tube A is filled to the mark U with a mixture of 10 c.c. of a 1 per cent. solution of serum-albumin in 0.4 per cent. of hydrochloric acid and 5 c.c. of filtered gastric juice. The second tube, B, which is the standard, is likewise filled to the mark U, but 0.5 gramme of pepsin is added to the serum solution, instead of the gastric juice. The third tube, C, contains merely a mixture of the serum solution and 5 c.c. of water. After the tubes have been kept in the thermostate for one hour, at a temperature of 37° C. Esbach's reagent is added to each tube to the mark R. After standing for twenty-four hours the amount of precipitated albumin is read off, and the difference between that in tube A and tube C compared with that in tube B.

Mett's Method.[2]—Satisfactory comparative results can also be obtained with the method suggested by Mett. Capillary glass tubes are prepared measuring from 1 to 2 mm. in diameter. They are filled with white of egg, which is coagulated in the tubes at a temperature of 95° C. The tubes are then cut into pieces from 1 to 2 cm. long and placed in the digestive mixture to be examined. The length of the column digested in a given length of time serves as a measure of the digestive power of the specimen examined. In practice this column should be measured in millimeters with the aid of a magnifying-glass. The calculation of the corresponding amount of ferment is based upon the law of Schütz and Borissow, viz., that the corresponding amounts of ferment in two solutions bear the same ratio toward each other as the square of the number of millimeters of the column of egg albumin which has been dissolved in the same length of time.

[1] Hammerschlag, Wien. med. Presse, 1894, vol. xxxv. p. 1654.

[2] Mett's method is described by Pawlow, die Arbeit d. Verdauungsdrüsen. Translated into German from the Russian by A. Walther, Wiesbaden, 1898.

Example.—The gastric juice of a normal individual is procured at the height of digestion after giving Ewald's test breakfast. The tube is digested for thirty minutes; at the end of this time the height of the column of albumin digested measures 3 mm. Then the stomach contents of a second individual are obtained (the patient's) and similarly treated; in this case the column of digested albumin measures 2 mm. The corresponding amounts of ferment are then as 9 is to 4.

Of Pepsinogen.—In order to estimate the amount of pepsinogen the method of Boas may be employed. To this end, the gastric juice is diluted with distilled water in varying proportions, such as 1 : 5, 1 : 10, 1 : 20, etc. A known quantity of coagulated albumin is added to each specimen, as also 1 or 2 drops of an officinal solution of hydrochloric acid, for each 10 c.c. employed. These tubes are kept at a temperature of from 37° to 40° C., when the degree of dilution is noted at which the bit of egg-albumin is still dissolved. The greater the degree of dilution at which digestion still takes place, the greater the amount of pepsin or of its zymogen present.

Both Hammerschlag's and Mett's method can also be applied in the estimation of pepsinogen, after rendering the gastric contents acid, with hydrochloric acid, to the extent of from 1 to 2 pro mille.

If it is desired to exclude definitely the presence of pepsin and pepsinogen in the stomach, the method of Jaworski should be employed. To this end, about 200 c.c. of a decinormal solution of hydrochloric acid are poured into the stomach through a tube and aspirated after one-half hour. If the fluid removed contains no pepsin, the absence of both the enzyme and its zymogen may be inferred.

The Milk-curdling Ferment and its Zymogen, viz., Chymosin and Chymosinogen.—A great deal of what has been said above regarding pepsin and its zymogen also holds good for chymosin and its pro-enzyme. The pro-enzyme thus also appears to be formed by the cell, as a neutral aqueous extract of the mucous membrane of the stomach does not, as a rule, contain the ferment, but the zymogen, the ferment resulting only when the latter is treated with a free acid. It differs from pepsin in that it can exert its physiological activity in feebly acid, neutral, and even feebly alkaline solutions. Exposure of an active solution of chymosin to gastric juice containing 3 pro mille of free hydrochloric acid, moreover, at a temperature of from 37° to 40° C., leads to its destruction.

Its specific action is exerted upon milk, or lime-containing solutions of caseïn, which are coagulated in neutral or feebly alkaline solutions.

In this connection it is important to note that the addition of a few cubic centimeters of a solution of calcium chloride, or any other

soluble lime salt, results in a transformation of the zymogen into the physiologically active ferment, and that hydrochloric acid, while it normally causes such transformation, is not absolutely necessary in the presence of calcium chloride.

Under physiological conditions chymosin and its zymogen are always present in the gastric juice. In disease the inferences that may be drawn from a quantitative estimation of the ferment and its zymogen have been well formulated by Boas,[1] to whom we are especially indebted for a great deal of valuable information in this connection:

1. Notwithstanding the absence of free hydrochloric acid, chymosin may be present, although in minimal traces—*i. e.*, demonstrable with a dilution of from 1 : 10 to 1 : 20 (see method below).

2. In the absence of free hydrochloric acid the zymogen may still be present in normal amounts—*i. e.*, demonstrable with a dilution of from 1 : 100 to 1 : 150. The presence of the zymogen, especially when repeatedly observed, probably always permits of the conclusion that we are not dealing with an organic disease of the stomach, but with a neurosis or a hyperæmic condition of the mucous membrane referable to disease of other organs.

3. The zymogen may occur in moderately diminished amount, 50 per cent. only being present. This is usually owing to the existence of a gastritis which has not reached its highest degree of severity. The nearer the amount of zymogen approaches the normal, the greater will be the probability of an ultimate recovery under suitable treatment.

4. The amount of the zymogen is greatly diminished (dilutions of 1 : 10 to 1 : 25 yielding a negative result) or may be absent altogether. In cases of this kind a severe and usually incurable gastritis exists, either primary or occurring secondarily to carcinoma, amyloid degeneration, etc.

5. In conditions 1, 2, and 3, the re-establishment of the secretion of hydrochloric acid may be attempted with some prospect of success by means of stimulating remedies.

These conclusions are based upon the employment of Ewald's test-breakfast, and cannot be applied to observations made after other test-meals, without previous studies in this direction.

Testing for the presence of chymosin and its zymogen, moreover, is of decided value in cases in which alkaline material is vomited, and where we may be called upon to decide whether this contains constituents of the gastric juice or not.

Tests for Chymosin and Chymosinogen.—TEST FOR THE ENZYME.—Five to 10 c.c. of milk are treated with from 3 to 5 drops of the filtered gastric juice and kept at a temperature of from

[1] Boas. Centralbl. f. d. med. Wiss., 1887, vol. xxv. p. 417; and Zeit. f. klin. Med., 1888, vol. xiv. p. 240. See also J. Friedenwald, Med. News, 1895.

37° to 40° C. for ten to fifteen minutes. If coagulation occurs during this time, it may be concluded that the enzyme is present.

Test for the Zymogen.—The milk is treated with 10 c.c. of the filtered and feebly alkalinized gastric juice and with 2 or 3 c.c. of a 1 per cent. solution of calcium chloride. The mixture is kept at a temperature of from 37° to 40° C., when in the presence of the zymogen the formation of a thick cake of caseïn will be observed to occur within a few minutes.

Quantitative Estimation.—Of the Enzyme.—The method is based upon the fact that on gradually diluting a specimen of gastric juice a point is finally reached at which a chymosin reaction can no longer be obtained, the value being, of course, a relative one. Under physiological conditions a positive reaction can still be observed with a degree of dilution varying between 1 : 30 and 1 : 40.

The gastric juice is neutralized with a very dilute solution of sodium hydrate. Tubes are then prepared containing from 5 to 10 c.c. of the gastric juice, diluted in the proportion of 1 : 10, 1 : 20, 1 : 30, etc., to which an equal amount of neutral or amphoteric milk is added. The tubes, properly labelled, are kept at a temperature of from 37° to 40° C., and the degree of dilution noted at which coagulation still occurs.

Of the Zymogen.—The gastric juice is rendered feebly alkaline and tubes are prepared containing equal amounts of milk and gastric juice, the latter variously diluted, as above directed; the examination is then carried on in the same manner. Normally a positive reaction is obtained with a dilution varying between 1 : 150 and 1 : 100. Allowance must, of course, be made for the amount of fluid which is added during the process of neutralization.

The Products of Gastric Digestion.

Digestion of the Native Albumins.—The first step in the process of albuminous digestion in the stomach is one of swelling, which may be observed when a flake of fibrin, for example, is placed in gastric juice and the temperature maintained between 37° and 40° C. Very soon simple solution takes place, which is followed by the process of "denaturization," as Neumeister terms it, in which the native albumins are transformed into acid albumins or syntonins, owing to the continued activity of the hydrochloric acid and pepsin. The pepsin, however, acts only as an adjuvant to the acid, and hydrochloric acid alone is capable of effecting the same result. But while in the absence of pepsin more concentrated solutions of the acid and a higher temperature are required, the temperature of the body and the amount of hydrochloric acid secreted by the stomach are sufficient when pepsin is present. Pepsin, in the absence of free hydrochloric acid, is perfectly inert.

The "denaturization" of the native albumins is followed by a

splitting up of the albuminous molecule and a process of hydration. the so-called primary albumoses being the first products thus formed. During the further process of digestion the deutero-albumoses then result, and from these the peptones, to which, in contradistinction to the peptones formed during the process of *pancreatic* digestion, the term *amphopeptone* has been applied by Kühne.

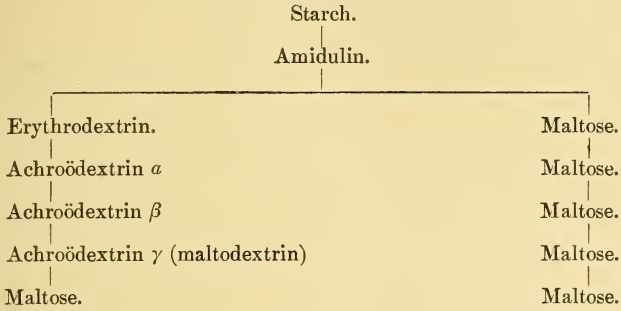
Digestion of the Proteids.—The digestion of caseïn, which belongs to the class of nucleo-albumins, differs from the process described. The caseïn of the milk is present in solution as a neutral calcium salt, and as it has the character of a polybasic acid, calcium chloride and the corresponding acid caseïn salt will result in the presence of the hydrochloric acid of the stomach ; still later, when more hydrochloric acid has been secreted, insoluble caseïn, as such, will be found. While the acid is thus capable of causing the precipitation of caseïn, it has also been shown that the same result may be reached in the absence of hydrochloric acid. According to Hammarsten, this is brought about in consequence of the hydrolytic action on the part of the chymosin, the calcium salt of paracaseïn (cheese), and a small amount of an albumose-like posset-albumin being formed. This latter process is now supposed to take place in the stomach after the hydrochloric acid has previously transformed the neutral into the acid caseïn salt. When this stage is reached the paracaseïn is decomposed into an albumin and an insoluble nuclein. The albumin is then further digested as described ; a hetero-albumose, however, does not result. The remaining proteids, such as hæmoglobin, glucosides, etc., are similarly acted upon by the gastric juice, and are first split up into the corresponding albumins and their pairlings. The albuminous radicles are then digested, as described.

Digestion of the Albuminoids.—Of the albuminoids, only collagen and elastin undergo digestion in the stomach, gelatoses and elastoses being formed during the process, while keratin passes off undigested. Hetero-albumoses, however, are formed from neither collagen nor elastin, but merely proto-albumoses, which in turn are transformed into deutero-albumoses, and these into peptone.

Digestion of the Carbohydrates.—The secretion of the stomach itself is not capable of digesting carbohydrates. There appears to be no doubt, however, that a transformation of starches into sugar takes place during the earlier stages of digestion. This is owing to the continued action of the ptyalin of the saliva (see page 199) in the stomach, which proceeds until the amount of hydrochloric acid secreted reaches 0.01 per cent. or more, it being remembered that the transformation of starches into sugar takes place best in a neutral or feebly alkaline medium.

The question whether or not a diastatic ferment occurs in the mucus secreted by the stomach itself is unimportant, as cases have but rarely been observed in which there was an absence of ptyalin from the saliva.

As indicated in the chapter on the Saliva, a number of intermediary products are formed in the transformation of starch into sugar, of which an idea may be had from the accompanying table:



In the mouth this transformation is effected very rapidly in the case of certain starches, such as corn-starch and rye-starch, and it is possible to demonstrate the presence of sugar after from two to six minutes. Potato-starch, on the other hand, requires a much longer time, viz., from two to four hours. This difference is entirely dependent upon the varying degree of resistance offered to the action of the saliva by the enclosing envelope of cellulose, as is apparent from the fact that a paste made from potatoes is digested just as rapidly as one made from rye.

For practical purposes, the digestion of carbohydrates in the stomach may be disregarded as insignificant.

Fats are not digested in the stomach.

From the above considerations it is apparent that under physiological conditions a mixture of various products is met with in the stomach at the height of digestion, and it might be expected that from a preponderance of the one over the other definite and valuable conclusions as to the digestive power of the organ could be reached. While this is true in a certain sense, the quantitative methods of analysis that would have to be employed in order to obtain definite data are as yet too complicated for the purposes of the clinician, and from the simple qualitative tests not much information can be derived. The recognition of the presence of peptones would thus merely indicate the presence of hydrochloric acid and pepsin in a general way, as peptones may be formed in the absence of hydrochloric acid and in the presence of organic acids, which may be found in pathological conditions. A portion of the albumin of milk, eggs, meat, etc., is, moreover, already peptonized during the process of boiling. It is not surprising then that peptones may be demonstrated in practically every specimen of gastric contents.

A large amount of syntonin and primary albumoses in the presence

of a feeble peptone-reaction must, of course, be regarded as abnormal, pointing to a defective secretion of either hydrochloric acid or the enzymes, or of both. The same may be said to hold good when a pronounced peptone-reaction disappears upon the removal of syntonin and the primary albumoses.

So far as the examination for the products of carbohydrate digestion is concerned, it may be stated, as a general rule, that in the presence of a normal amount of hydrochloric acid erythrodextrin can usually be demonstrated toward the end of gastric digestion, while achroödextrin is nearly always obtained at that time when free hydrochloric acid is absent, so that the tests for the presence of these two bodies may be regarded as roughly indicating the presence or absence of free hydrochloric acid. Boas draws attention to the fact, however, that ptyalin may, at times, though rarely, be absent, when conclusions drawn from these tests as to the presence of hydrochloric acid would be erroneous.

The tests for sugar in the gastric juice do not furnish any information of practical value.

Analysis of the Products of Albuminous Digestion.

In order to separate the various bodies referred to from each other the following procedure may be employed:

The filtered gastric contents are carefully neutralized with a dilute solution of sodium hydrate, using litmus-paper to determine the reaction; a small drop of the mixture is placed upon the paper from time to time during the addition of the sodium hydrate until no change in color is produced either on the red or the blue paper. If syntonin is present, it will be precipitated, and can be collected on a small filter. Upon the addition of an excess of dilute acid or an alkali this precipitate will again be dissolved. The filtrate is feebly acidified by the addition of a few drops of a very dilute solution of acetic acid, treated with an equal volume of a saturated solution of common salt, and brought to the boiling-point. Any native albumin that may be present in solution is thus coagulated and can be filtered off on cooling. In the filtrate the albumoses and peptones remain. The presence of the former may be demonstrated by adding a few drops of nitric acid to a specimen, when a precipitate will form which dissolves upon the application of heat, and reappears on cooling; if necessary, the specimen may be diluted.

Should the deutero-albumoses of vitellin or myosin be present, however, this test yields a negative result, and a precipitate only occurs when the solution, acidified with nitric or acetic acid, is completely saturated with sodium chloride.

The presence of primary albumoses may be established by adding pieces of rock-salt to the neutral solution, when a precipitate occurs.

The albumoses may roughly be separated from the peptones by saturating the acidified filtrate just obtained with pulverized ammonium sulphate, whereby the albumoses are precipitated almost entirely. A small portion of deutero-albumoses, however, which resulted from the proto-albumoses, remains in solution and passes into the filtrate, which also contains all of the amphopeptone. In the filtrate this may be demonstrated as follows: a concentrated solution of sodium hydrate is added until all the ammonium sulphate has been transformed into sodium sulphate, and a slight excess of the hydrate is present; care should be had, however, that the temperature does not rise too high, by immersion in cold water. The sodium sulphate, which separates out during this process, is allowed to settle. A 2 per cent. solution of cupric sulphate is then carefully added drop by drop, to a specimen of the supernatant fluid, when in the presence of peptones a rose to a purplish-red color will develop.

To obtain the peptones, the filtrate is diluted with an equal volume of water, neutralized, and then treated with a solution of tannic acid, care being taken to avoid an excess, as otherwise the peptone precipitate is partly dissolved.[1]

Tests for the Products of Carbohydrate Digestion.

Starch may be recognized by the fact that it strikes a blue color with a solution of iodo-potassic iodide, while the same solution gives a violet or mahogany brown with erythrodextrin. To this end, it is only necessary to add a drop or two of Lugol's solution to a few cubic centimeters of the filtered gastric juice. The presence of achroödextrin may be inferred if no change in color occurs upon the addition of the reagent.

Maltose and dextrose, which both react with Fehling's solution and undergo fermentation, differ from each other in the fact that the former does not reduce *Barfoed's reagent* on boiling. This is prepared by adding 1 per cent. of acetic acid to a 0.5 to 4 per cent. solution of cupric acetate. The rotatory power of maltose is about three times as strong as that of dextrose; $(\alpha)\ D = 150.4$, as compared with 52.5.

Lactic Acid.

Mode of Formation and Clinical Significance.—It was formerly thought that the acidity of the gastric juice was referable to the presence of lactic acid, as this can always be demonstrated in the beginning of the process of digestion. The hydrochloric acid

[1] For a more detailed account of the chemistry of digestion and the analysis of the resulting products, see C. E. Simon, Physiological Chemistry, Lea Bros. & Co., 1901.

was then supposed to result from the action of the lactic acid upon the chlorides of the food. That this view is erroneous C. Schmidt[1] succeeded in demonstrating beyond a doubt, as has been shown on page 217. An explanation of the presence of lactic acid suggested itself when Miller found that in the mouth various bacteria normally occur which are capable of forming lactic acid from sugar, and that from the gastric contents a number of bacteria can be isolated which are capable of causing acid fermentation in sugar-containing media. There would, hence, be nothing surprising in the constant occurrence of lactic acid, as the two principal factors necessary for its formation are always present after the ingestion of an ordinary meal, viz., carbohydrates and bacteria capable of causing lactic acid fermentation. The absence of the lactic acid during the later stages of digestion was, furthermore, explained by the fact that lactic acid fermentation ceases in the presence of from 0.7 to 1.6 pro mille of hydrochloric acid—*i. e.*, in the presence of amounts of hydrochloric acid which are found in the normal gastric juice.

The normal occurrence of lactic acid in the stomach was, until recently, regarded as an established fact. But at this stage Martius and Lüttke, employing the method already described, found "that the accurately determined curve of acidity referable to hydrochloric acid coincided in all respects, even at the beginning of the process of digestion, with the curve referable to the total acidity," so that lactic acid as a physiological constituent could not have been present. Recent researches of Boas,[2] moreover, appear to prove beyond a doubt that in physiological conditions no appreciable amounts of lactic acid are formed during the process of digestion, and that the lactic acid found after an ordinary meal has been introduced into the stomach as such. That lactic acid is actually present in the various kinds of bread has definitely been proved, and it is, hence, not permissible to make use of any test-meal containing lactic acid when the question as to its formation in the stomach is to be considered. For these reasons Boas suggests the use of simple oatmeal-soup to which salt only has been added. For practical purposes this is probably not always necessary, as the small amount of lactic acid found after Ewald's test-breakfast may usually be disregarded; an increased amount can be referred directly to pathological conditions.

The fact that the lactic acid disappears, or is at least no longer demonstrable, at the height of digestion, Boas refers to a resorption or a carrying-off of the acid introduced, on the one hand, or to an interference of the hydrochloric acid with the delicacy of the reagent

[1] Loc. cit.

[2] J. Boas, "Ueber d. Vorkommen v. Milchsäure im gesunden u. kranken Magen," Zeit. f. klin. Med., 1894, vol. xxv. p. 285.

usually employed—*i. e.*, Uffelmann's reagent—on the other. Pathologically the same rule may be said to hold good, as Boas was unable to demonstrate its presence after the exhibition of his test-meal in the most diverse diseases of the stomach, viz., chronic gastritis, atony and dilatation referable to myasthenia, or pyloric stenosis following ulcer, etc. Mere traces, which were occasionally observed, are of no significance, and possibly referable to lactic acid fermentation having taken place in the mouth. In all the cases examined, moreover, no organic acids could be demonstrated by the method of Hehner-Seemann (see page 255).

It is apparent then that notwithstanding stagnation of the gastric contents and the absence of free hydrochloric acid in normal amounts, lactic acid is not necessarily formed in the stomach, even in the presence of carbohydrates. In only one disease of the stomach was lactic acid found in notable quantities, viz., in carcinoma. This observation is in accord with the fact that Uffelmann's test here yields a marked reaction—*i. e.*, a deep-lemon or a canary-yellow color—even upon the addition of but a few drops of the gastric juice, while in the benign affections only a pale-yellow, brownish, or grayish color is obtained.

Boas' test-meal should be given the evening before the examination, the stomach having previously been washed free from all remnants of food; the remaining contents are obtained the next morning.

In an analysis of fourteen cases of carcinoma Boas was able to demonstrate the presence of lactic acid in amounts varying between 1.22 and 3.82 pro mille in all cases but one, while in other diseases after the ingestion of Ewald's test-breakfast only 0.1 to 0.3 pro mille could be obtained.

Unfortunately, recent investigations have shown that notable amounts of lactic acid may also be found in gastric anadeny, and in cases of dilatation referable to benign causes. Such cases, however, are rare, and it may safely be stated that the presence of large amounts of lactic acid will almost invariably justify the diagnosis of carcinoma of the stomach.[1] Lactic acid may, however, *not* be found in cases of pyloric carcinoma so long as any hydrochloric acid is secreted; and may be present, on the other hand, in cases of stenosing gastritis referable to benign causes.

That stagnation of the gastric contents and the absence of free hydrochloric acid alone are not capable of causing the formation of lactic acid has been seen, and it is, hence, difficult to explain why in carcinoma practically only lactic acid fermentation should occur.

[1] J.H. de Jong, "Der Nachweis d. Milchsäure u. ihre klinische Bedeutung," Arch. f. Verdauungskrank., vol. ii. p. 53. J. Friedenwald, "The Significance of the Presence of Lactic Acid in the Stomach." N. Y. Med. Jour., 1895. Rosenheim u. Richter, "Ueber Milchsäurebildung im Magen," Zeit. f. klin. Med., vol. xxviii. p. 505.

Whether the malignant growth itself must be regarded as one of the principal factors in this connection, as Boas suggests, must still remain an open question.

Owing to the interest which attaches to this subject, it may not be out of place to refer briefly to the following observation of Koch: In a case in which ulcer of the stomach existed, the hydrochloric acid suddenly disappeared and gave place to lactic acid, which then steadily increased in amount from week to week. A tumor could not be demonstrated on physical examination. Soon after, the patient died, and at the autopsy a carcinoma of the stomach was found upon the base of the pyloric ulcer. *An exploratory operation should hence be made whenever notable amounts of lactic acid can repeatedly be demonstrated in the stomach contents after the ingestion of Boas' test-meal.* Negative results, however, do not exclude the existence of carcinoma.

The formation of lactic acid from starch may be represented by the following equations:

(1) $2C_6H_{10}O_5 + H_2O = C_{12}H_{22}O_{11}$ (milk-sugar).
(2) $C_{12}H_{22}O_{11} + H_2O = 2C_6H_{12}O_6$ (glucose).
(3) $2C_6H_{12}O_6 = 4C_3H_6O_3$ (lactic acid).

It should, finally, be mentioned that only that form of lactic acid which results from fermentative processes is of interest in this connection, and not the sarcolactic acid contained in meat.

Tests for Lactic Acid.—For the reasons indicated, Boas' test-meal (see page 212) should be employed whenever it is desired to test for lactic acid in the gastric contents. If the case under examination shows well-marked symptoms of stagnation, the stomach should be washed out completely in the evening, the soup then given, and the gastric contents procured the next morning, before any food or liquid is taken. Otherwise the test-meal may be given in the morning on an empty stomach, without previous lavage, and the contents examined one hour later.

Uffelmann's Test.[1]—Heretofore Uffelmann's reagent was quite commonly employed in testing for lactic acid, but everyone who has had occasion to make frequent use of this reagent in clinical work must have been struck with the uncertainty of the results so often obtained. In a large majority of the cases thus examined, particularly if Ewald's test-breakfast is employed, a characteristic reaction —*i. e.*, the occurrence of a lemon or canary-yellow color—is not seen, notwithstanding the presence of lactic acid, but a pale-yellow, brownish, grayish-white, or even gray color is obtained instead, often leaving in doubt whether lactic acid is present or not. Aside from doubtful results, the value of the test is greatly diminished by the

[1] Uffelmann, Deutsch. Arch. f. klin. Med., 1880, vol. xxvi.; and Zeit. f. klin. Med., vol. viii. p. 392.

fact that glucose, acid phosphates, butyric acid, and alcohol give the same reaction, and that in the presence of such amounts of hydrochloric acid as are found at the height of normal digestion lactic acid is not indicated by the reagent. All these difficulties have long been appreciated, and in order to obviate at least some of them it was proposed to apply the test to an aqueous solution of the ethereal extract of the gastric contents:

To this end, 5 or 10 c.c. of the filtered gastric juice are extracted by shaking with from 50 to 100 c.c. of neutral sulphuric ether in a stoppered separating-funnel for about twenty or thirty minutes; the ethereal extract is then evaporated on a water-bath or the ether distilled off (*no flame*). The residue is taken up with from 5 to 10 c.c. of distilled water, and tested as follows: three drops of a saturated aqueous solution of ferric chloride are mixed with three drops of a concentrated solution of pure carbolic acid and diluted with water until an amethyst-blue color is obtained; to this solution a portion of the ethereal extract is added, when in the presence of only 0.1 per cent. of lactic acid a lemon or canary-yellow color is obtained.

Kelling's Method.[1]—Five or 10 c.c. of gastric juice are diluted with from ten to twenty volumes of water and treated with one or two drops of a 5 per cent. aqueous solution of ferric chloride. In the presence of lactic acid a distinct greenish-yellow color is seen if the tube is held to the light. This test is more reliable than that of Uffelmann, as a positive reaction is obtained only in the presence of lactic acid.

Strauss' Method.[2]—Instead of evaporating the ether as in the above method, the ethereal extract may be directly examined by shaking with a freshly prepared solution of ferric chloride, as suggested by Fleischer. Making use of this principle, Strauss has constructed an apparatus (Fig. 46) which may be found very convenient, and which permits of roughly determining the amount of lactic acid present. The instrument is essentially a separating-funnel of 30 c.c. capacity, bearing two marks, of which the one corresponds to 5 c.c., the other to 25 c.c. The apparatus is filled with gastric juice to the mark 5, when ether is added to the 25 c.c. line. After shaking thoroughly, the *separated* liquids are allowed to escape by opening the stopcock until the 5 c.c. mark is reached. Distilled water is then added to the 25 mark, and the mixture treated with two drops of the officinal tincture of ferric chloride, diluted in the proportion of 1 : 10. Upon shaking, the water will assume an intensely green color if more than 1 pro mille of lactic acid is present, while a pale green is obtained in the presence of from 0.5 to 1

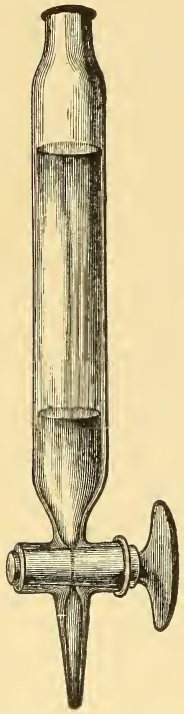
[1] G. Kelling, "Rhodan im Mageninhalt; Zugleich ein Beitrag z. Uffelmann'schen Milchsäurereagens," Zeit. f. physiol. Chem., vol. xviii.

[2] H. Strauss, "Ueber eine Modifikation d. Uffelmann'schen Reaktion," Berlin. klin. Woch., 1895, No. 37.

pro mille. The tincture of iron should be kept in a dark-colored dropping-bottle of about 50 c.c. capacity.

It will be observed that only large amounts of lactic acid, which alone are of importance from a diagnostic point of view, are indicated by the apparatus. Small amounts, as those introduced with Ewald's test-breakfast, or referable to lactic acid fermentation in the mouth, are not indicated, so that confusion as to the presence or absence of the acid can never arise.

Fig. 46.



Strauss' apparatus for the approximative estimation of lactic acid.

Boas' Method.[1]—In doubtful cases the following method should be employed, as with it, and following the exhibition of Boas' test-meal, all possible errors can be avoided. *The stomach must, however, be washed perfectly clean before the test-meal is introduced.* It is my belief that some of the positive results which have been obtained in other diseases than carcinoma are referable to neglect in this particular point. Aldehyde is not infrequently found in the stomach contents when sarcinæ are present in large numbers, and may be mistaken for lactic acid, as I discovered to my regret not long ago.

Principle of the Method.—When a solution of lactic acid is treated with a strong oxidizing agent and heated, the lactic acid is decomposed into acetic aldehyde and formic acid, according to the equation

$$\underset{\text{Lactic acid.}}{CH_3-CH(OH)-CO.OH} = \underset{\text{Acetic aldehyde.}}{CH_3.CHO} + \underset{\text{Formic acid.}}{H.CO.OH.}$$

Practically, then, the test for lactic acid resolves itself into a test for acetic aldehyde, which can readily be recognized by testing with various reagents, such as an alkaline solution of iodo-potassic iodide, Nessler's reagent, and others. Nessler's reagent is prepared as follows: 2 grammes of potassium iodide are dissolved in 50 c.c. of water and treated with mercuric iodide while heating, until some of the latter remains undissolved. Upon cooling, the solution is diluted with 20 c.c. of water. Two parts of this solution are then treated with 3 parts of a concentrated solution of potassium hydrate; any precipitate that may have formed is filtered off, and the reagent kept in a well-stoppered bottle. When aldehyde is added to such a solution a yellowish-red or red precipitate results, the exact color depending upon the amount of aldehyde present. One part of the

[1] Boas, Deutsch. med. Woch., 1893, No. 39; and Münch. med. Woch., 1893, No. 43.

aldehyde may still be recognized when diluted with 40,000 parts of water.

With an alkaline solution of iodo-potassic iodide, aldehyde in a dilution of 1 : 20,000 will still produce a cloudiness, referable to the formation of iodoform, which is readily recognized by its characteristic odor (Lieben's test for acetone).

METHOD.—The filtered gastric juice is tested for the presence of free acids with Congo-red (see page 225) If present, from 10 to 20 c.c. are evaporated to a syrup on a water-bath, after the addition of an excess of barium carbonate, while the latter is unnecessary in the absence of free acids. The syrup is treated with a few drops of phosphoric acid, and the carbon dioxide removed by bringing it to the boiling-point once only, when it is allowed to cool and extracted with 100 c.c. of neutral sulphuric ether (free from alcohol), by shaking for half an hour. The layer of ether is poured off after half an hour, the ether is evaporated (*no flame*), the residue taken up with 45 c.c. of water, shaken and filtered, and finally treated with 5 c.c. of sulphuric acid and a pinch of manganese dioxide in an Erlenmeyer flask. This is closed with a perforated stopper carrying a glass tube bent at an obtuse angle, the longer limb of which passes into a narrow glass cylinder containing from 5 to 10 c.c. of Nessler's reagent or a like quantity of an alkaline solution of iodo-potassic iodide. If heat is now carefully applied, the aldehyde, formed by the oxidation of the lactic acid with manganese dioxide and sulphuric acid, passes over when the boiling-point is reached, and causes the precipitation of yellowish-red aldehyde of mercury in the tube containing the Nessler's reagent, or of iodoform if the alkaline solution of iodine is employed.

Quantitative Estimation of Lactic Acid according to Boas' Method.[1]—The principle already set forth also applies to the quantitative estimation of lactic acid.

Solutions required :

1. A one-tenth normal solution of iodine.
2. A one-tenth normal solution of sodium thiosulphate.
3. Hydrochloric acid (sp. gr. 1.018).
4. A potassium hydrate solution (56 : 1000).
5. Starch solution.

Preparation of these solutions :

1. A normal solution of iodine should contain 126.53 (molecular weight of iodine) grammes of iodine in the liter, and a one-tenth normal solution, hence 12.6 grammes. In order to dissolve the iodine 25 grammes of potassium iodide are dissolved in about 200 c.c. of distilled water, when the 12.6 grammes of resublimed iodine are added. This solution is then diluted with distilled water to the 1000 c.c. mark, and requires no further correction.

[1] Loc. cit., p. 187.

2. The one-tenth normal solution of sodium thiosulphate is prepared as described in the chapter on Acetone (see Urine). When treated with 1 gramme of ammonium carbonate pro liter it will retain its titre almost indefinitely.

3. Preparation of the starch solution: 5 grammes of starch are dissolved in 900 c.c. of water by heating, when 10 grammes of zinc chloride in 100 c.c. of water are added.

METHOD.—Ten to 20 c.c. of the filtered gastric juice are first treated as indicated above, viz., evaporated to a syrup after the addition of barium carbonate if free acids are present. A few drops of phosphoric acid are added, the carbon dioxide driven off by boiling, and the residue extracted, on cooling, with 100 c.c. of ether *free from alcohol;* the ether is evaporated after separation, the residue taken up with 45 c.c. of distilled water, and treated with manganese dioxide and sulphuric acid. The flask is closed by a doubly perforated stopper; through one aperture a bent tube passes to the distilling-apparatus, and a straight tube provided with a piece of rubber tubing, clamped off, through the other. The latter should dip well down into the liquid, and serves for passing a current of air through the solution when the distillation is completed. The mixture is distilled until about four-fifths of the contents have passed over, *excessive heat being carefully avoided,* as otherwise the aldehyde will be decomposed, according to the equations:

$$(1)\quad \underset{\text{Lactic acid.}}{CH_3 - CH(OH) - CO.OH} = \underset{\text{Aldehyde.}}{CH_3.CHO} + \underset{\text{Formic acid.}}{HCOOH}.$$

$$(2)\quad \underset{\text{Aldehyde.}}{CH_3.CHO} + \underset{\text{Formic acid.}}{HCOOH} + 2O = \underset{\text{Acetic acid.}}{CH_3.COOH} + CO_2 + H_2O.$$

To the distillate, which is best received in a high Erlenmeyer flask, well stoppered, 20 c.c. of the one-tenth normal solution of iodine are added, mixed with 20 c.c. of the 5.6 per cent. solution of potassium hydrate. The mixture is shaken thoroughly and allowed to stand for a few minutes. In order to liberate the iodine not used in the reaction, 20 c.c. of hydrochloric acid are added, and the excess of iodine determined by titration with the one-tenth normal solution of sodium thiosulphate. The titration is carried almost to the point of decolorization, when a little starch solution is added; the mixture is then titrated until the blue color has disappeared. The number of cubic centimeters of the one-tenth normal solution employed, viz., 20, minus the number of cubic centimeters of the one-tenth normal solution of sodium thiosulphate, will then indicate the number of cubic centimeters of the former required for the formation of iodoform, viz., the amount of lactic acid present in 10 or 20 c.c. of gastric juice, as the case may be. As 1 c.c. of the one-tenth normal solution of iodine has been found to indicate the presence of

0.003388 gramme of lactic acid, it is only necessary to multiply the number of cubic centimeters used by this figure, and the result by 10, in order to obtain the percentage.

The method described is reliable and sufficiently accurate for clinical purposes. At the same time it may be said that no more time is required than in the ordinary quantitative estimation of sugar by means of Fehling's method, or of hydrochloric acid according to the method of Martius and Lüttke.

Boas' Rapid Method.—This method is less accurate than the preceding one, but may be advantageously employed in the absence of the various reagents necessary with the former. Ten c.c. of filtered gastric juice are treated with a few drops of dilute sulphuric acid, and the albumin present removed by heat. The filtrate is evaporated to a syrup on a water-bath, water added to the original amount, and this again evaporated to a small volume, fatty acids being thereby removed. The lactic acid remaining is now extracted with ether (200 c.c. for every 10 c.c. of gastric juice); the ether is evaporated, the residue taken up with water and titrated with a one-tenth normal solution of sodium hydrate, using phenolphthalein as an indicator. As 40 parts by weight of sodium hydrate (molecular weight) combine with 90 parts by weight of lactic acid (molecular weight), and as 1 c.c. of the one-tenth normal solution of sodium hydrate contains 0.004 gramme of sodium hydrate, the corresponding amount of lactic acid is found from the equation: $40 : 90 : : 0.004 : x$; $40\ x = 0.360$; $x = 0.009$. The value of 1 c.c. of the one-tenth normal solution in terms of lactic acid is thus 0.009. By multiplying the number of cubic centimeters used by this figure, the amount of lactic acid present in 10 c.c. of gastric juice is ascertained. The result multiplied by 10 indicates the percentage.

The Fatty Acids.

Mode of Formation and Clinical Significance.—Unless much milk or carbohydrates have been ingested, fatty acids do not occur in the gastric contents under physiological conditions, and it would appear from the researches of Boas [1] that their formation is intimately associated with that of lactic acid. After the exhibition of his test-meal (see page 212) he was unable to demonstrate their presence either in health or in various diseases of the stomach, such as chronic gastritis, atony or dilatation referable to benign causes, etc. In carcinoma, however, fatty acids, just as lactic acid, were quite constantly found.

That butyric acid can be derived from lactic acid has been demonstrated by Flügge, the reaction taking place according to the equation

$$2C_3H_6O_3 = C_4H_8O_2 + 2CO_2 + 4H.$$

[1] Loc. cit.

This observation is probably explained by the fact that most of the organisms causing butyric acid fermentation are anaërobic, while the Bacillus acidi lactici and the Oïdium lactis eagerly absorb oxygen.

Acetic acid fermentation, on the other hand, presupposes the presence of alcohol, whether this is introduced into the stomach as such or whether it results from the action of yeast (Saccharomyces cerevisiæ) upon sugar. The transformation of alcohol into acetic acid is represented by the equation

$$C_2H_5OH + 2O = C_2H_4O_2 + H_2O,$$

while the formation of alcohol during the process of fermentation from glucose is shown below:

$$C_6H_{12}O_6 + 2H_2O = 2C_2H_6O + 2H_2O + 2CO_2.$$

It is, hence, necessary, whenever acetic acid is met with in the gastric contents, to exclude the presence of alcohol, as only then is it permissible to refer its presence to stagnation and advanced decomposition of carbohydrates.

If the examination is confined to an analysis of the gastric contents obtained otherwise than after the exhibition of Boas' or Ewald's test-meal, the diagnosis of pyloric stenosis with dilatation is probably always justifiable in the presence of notable quantities of butyric acid and acetic acid, while the same after a previous washing-out of the stomach and the exhibition of Boas' test-meal would suggest carcinoma as the cause of the stenosis.

That butyric acid may occur in the gastric contents when butter or fats in general have been ingested is, of course, not surprising, and its presence then should be looked upon as a physiological occurrence. At the same time it should not be forgotten that butyric acid, just as lactic acid, may possibly have been formed in the mouth, and conclusions should, hence, only be drawn when such sources of error can be definitely excluded, and the amount found exceeds mere traces.

In conclusion, it may be said that in disease butyric acid is far more frequently encountered in the gastric contents than acetic acid, but the significance of the two, if alcoholism can be excluded, is the same.

Tests for Butyric Acid.—1. Butyric acid can usually be recognized by its odor alone, which is that of rancid butter. Often, however, it will be necessary to resort to more definite tests, such as the following:

2. Ten c.c. of filtered gastric juice are extracted with 50 c.c. of ether. The ether is evaporated and the residue taken up with a few cubic centimeters of water. If a trace of calcium chloride in substance is now added, the butyric acid will separate out in the form of oil-droplets, the nature of which is readily recognized by the pungent

odor. If, instead of adding calcium chloride, a slight excess of baryta-water is used, strongly refractive rhombic plates or granular, wart-like masses of barium butyrate are obtained upon evaporation.

3. Butyric acid may also be recognized by the peculiar odor of pineapple which develops when the dry residue of the ethereal solution is treated with a little sulphuric acid and alcohol. The reaction is due to the formation of butyl ethylate (Pineapple test).

Tests for Acetic Acid.—1. Like butyric acid, acetic acid can usually be recognized by its odor.

2. Ten c.c. of filtered gastric juice are extracted with ether. The ether is evaporated, the residue dissolved in a few drops of water, and accurately neutralized with a dilute solution of sodium hydrate, sodium acetate being formed. If to this a drop or two of a very dilute solution of ferric chloride is added, a dark-red color results in the presence of acetic acid. With silver nitrate a precipitate is obtained which is soluble in hot water.

Quantitative Estimation of the Fatty Acids.—Method of Cahn-Mehring, modified by McNaught.[1]—The total acidity is determined in 10 c.c. of filtered gastric juice. Another 10 c.c. are evaporated to a syrup, diluted with water, and similarly titrated. The difference between the two results will indicate the amount of fatty acids present.

Quantitative Estimation of the Organic Acids.—Method of Hehner-Seemann.[2]—This method is based upon the observation that if a certain amount of a one-tenth normal solution of sodium hydrate is added to organic acids and the mixture is evaporated and incinerated, the organic acids are decomposed, with the liberation of carbon dioxide, while their alkali is left behind in the form of a carbonate; this is then determined by titration with a one-tenth normal solution of hydrochloric acid. The amount of physiologically active hydrochloric acid can be estimated at the same time by deducting from the total acidity the acidity referable to organic acids.

Method.—Ten or 20 c.c. of filtered gastric juice are titrated with a one-tenth normal solution of sodium hydrate, evaporated to dryness, and incinerated, the application of heat being discontinued as soon as the ash has ceased to burn with a luminous flame. The residue is taken up with water and titrated with a one-tenth normal solution of hydrochloric acid. This is prepared by diluting 146 grammes of the concentrated acid (sp. gr. 1.14) with distilled water to about 900 c.c., when the solution is brought to the proper strength by comparing it with a one-tenth normal solution of sodium hydrate, according to directions given elsewhere. The number of cubic centimeters of the one-tenth normal solution of hydrochloric acid

[1] Cited by Boas, Diagnostik u. Therapie d. Magenkrankheiten, 2d ed., 1891, p. 140.

[2] Seemann, "Ueber d. Vorhandensein freier Salzsäure im Magen," Zeit. f. klin. Med., vol. v. p. 272.

employed multiplied by 0.00365 will indicate the amount of fatty acids in the 10 c.c. of gastric juice, in terms of hydrochloric acid; the percentage is ascertained by multiplying by 10 or 5, as the case may be. By deducting the number of cubic centimeters employed from that of the one-tenth normal solution of sodium hydrate, first used, the number of cubic centimeters of the latter required for the neutralization of the physiologically active hydrochloric acid is ascertained, and the amount determined by multiplying by 0.00365.

Gases.

The stomach always contains a certain quantity of gases which have partly been swallowed and partly have passed into the stomach from the duodenum. As fermentative processes in health occur only when carbohydrates or fats have been ingested, and then only to a slight degree, nitrogen, oxygen, and carbon dioxide are the only gases found during the process of albuminous digestion. As the oxygen swallowed is, moreover, largely absorbed by the blood, and two volumes of carbon dioxide are returned for one volume of oxygen, the presence of large amounts of the former and small amounts of the latter is readily explained. In an analysis of the gases contained in the stomach of a dog which had been fed on meat, Planer found the following proportions:

Carbon dioxide	25.2 vol. per cent.
Oxygen	6.1 " "
Nitrogen	68.7 " "

With a strict vegetable diet, on the other hand, hydrogen may also be found (Planer):

	Man.		Dog.
Carbon dioxide	20.79	33.83	32.9 vol. per cent.
Oxygen		0.37	0.8 " "
Nitrogen	72.50	38.22	66.3 " "
Hydrogen	6.71	27.58	

The presence of hydrogen is readily understood, if it is remembered that during the process of butyric acid fermentation hydrogen and carbon dioxide are formed. Lactic acid or acetic acid fermentation does not give rise to the formation of gases.

Marsh gas, CH_4, a product of the fermentation of cellulose, may also be found in pathological conditions, and is formed according to the equation

$$(C_6H_{10}O_5)_n + (H_2O)_n = 3(CO_2)_n + 3(CH_4)_n.$$

It is yet an open question whether marsh gas is formed in the stomach or passes into the stomach from the small intestine.

Such observations must, however, be regarded as rarities. In one case of this kind, examined by Ewald and Ruppstein,[1] in which alcohol, acetic acid, lactic acid, and butyric acid were found in the vomited material, an analysis of the gases gave the following result:

Carbon dioxide	20.6	vol. per cent.
Oxygen	6.5	" "
Nitrogen	41.4	" "
Hydrogen	20.6	" "
Marsh gas	10.8	" "

Traces of olefiant gas and of hydrogen sulphide were also found. It is curious to note that in this case the patient, who, according to his own statement, had a "vinegar-factory in his stomach on one day and gas-works on another day," was occasionally able to light the eructated gas at the end of a cigar-holder, where it burnt with a faintly luminous flame. McNaught has reported a similar case, in which the analysis furnished the following results: carbon dioxide, 56 per cent.; hydrogen, 28 per cent.; marsh gas, 6.8 per cent.; atmospheric air, 9.2 per cent.[2]

Ammonia and hydrogen sulphide are also at times met with; their presence is always due to albuminous putrefaction.

Boas[3] found that hydrogen sulphide is quite commonly present in cases of dilatation referable to benign causes, while it is almost always absent in carcinoma. He adds that it is never found when lactic acid is present. In acute gastritis it may be observed temporarily. In a number of cases of carcinoma I have never found hydrogen sulphide. In one case reported by Strauss the Bacillus coli communis was apparently concerned in its production.

To obtain a knowledge of the gases formed in the stomach during the process of digestion it is only necessary to fill an ordinary Doremus ureometer, or an Einhorn saccharimeter, with the unfiltered gastric contents, and to keep it at a temperature of from 37° to 40° C., when the evolution of gas can be followed closely and the necessary tests made. The presence of carbon dioxide is readily recognized by passing a small amount of sodium hydrate, in concentrated solution or in substance, into the tube, after the evolution has entirely ceased, when the fluid will rise. If other gases are present at the same time, they will remain after the carbon dioxide has been absorbed. Hydrogen sulphide is readily recognized by its odor and by the fact that it will color a piece of filter-paper, moistened

[1] Ewald, Arch. f. Anat. u. Physiol., 1874, p. 217.

[2] Kuhn, "Ueber Hefegährung und Bildung brennbarer Gase im menschlichen Magen," Zeit. f. klin. Med., vol. xxi.; and Deutsch. med. Woch., 1892, No. 49, and 1893, No. 15.

[3] Boas, "Ueber Schwefelwasserstoffbildung in Magenkrankheiten," Centralbl. f. inn. Med., 1895, No. 3; Deutsch. med. Woch., 1892, No. 49. Zawadzki, "Schwefelwasserstoff im erweiterten Magen," Centralbl. f. inn. Med., 1894, No. 50. Dauber, "Schwefelwasserstoff im Magen," Arch. f. Verdauungskrank., vol. iv. p. 4.

with a few drops of sodium hydrate and lead acetate a more or less pronounced brown or black. The test is conveniently made by filling a test-tube about half-full with the gastric contents and closing it with a cork stopper to which a strip of lead-paper, prepared as indicated, is fastened.

The eructation of gas formed in the stomach should not be confounded with the so-called *eructatio nervosa*, in which no gas is either eructated, or air simply enters the œsophagus and is expelled again with a loud, explosive noise. This may frequently be observed in neurasthenic and hysterical individuals, and is to a greater or less degree under the control of the will. It is hardly likely, however, that the physician will be called upon in the laboratory to differentiate between this form and that of true ructus, caused by fermentative processes taking place in the stomach. The gases brought up in the former condition are without odor or taste, and thus differ from those found in true dyspepsia.

Acetone.

The presence of acetone in the gastric contents in pathological conditions has repeatedly been observed, especially by v. Jaksch and Lorenz,[1] and it is curious to note that the latter was at times able to demonstrate larger quantities of the substance in the gastric contents than in the urine.

In the chapter on Acetonuria the relation existing between digestive diseases and the elimination of acetone will be dealt with more fully, but it may here be mentioned that in the *primary* diseases of the gastro-intestinal tract acetone is met with quite constantly in the gastric contents, while it is observed but rarely in the secondary forms, and never is seen in the gastric neuroses. This statement, however, is denied by Sovelieff, who claims to have found traces of acetone in only one case of nervous dyspepsia, while negative results were obtained in all other diseases of the stomach. I have repeatedly been able to demonstrate the presence of acetone in cases of carcinoma, and never have found it in neurotic conditions.

In order to test for acetone, the gastric contents are distilled after the previous addition of a small amount of phosphoric acid (1 : 1000), when the tests of Reynolds and Gunning (see Urine) are applied to the distillate. If both reactions furnish a positive result, the presence of acetone may be regarded as demonstrated. Denigès' test may also be employed, and can be applied to the filtered contents directly (see Urine).

Ptomaïns and Toxalbumins.

Remembering that ptomaïns and toxalbumins have been obtained directly from tainted meat, sausage, fish, clams, crabs, cheese, etc., it

[1] Lorenz, Zeit. f. klin. Med., 1891, vol. xix. p. 19.

is to be expected that these bodies may be met with in the gastric contents also. At the same time it may be mentioned that the stomach appears to possess the power of eliminating from the system poisons of this nature which are circulating in the blood. This is shown by the observations of Alt, who found that the water with which the stomach of an animal had been irrigated, after the subcutaneous injection of the poison of Pelias berus and Echidna arictans, or the direct bite of the snake, produced identical symptoms of poisoning when injected into another animal. It is interesting to note that with lavage of the stomach the poisoned animal recovered. Similar observations have been made in cholera Asiatica. Certain vegetable alkaloids, such as morphin, are also known to be eliminated to a large extent by the stomach. Of the nature of the ptomaïns and toxalbumins which may occur in the stomach, very little is known.[1]

Vomited Material.

Food-material.—The vomiting of large amounts of totally undigested meat two or three hours after its ingestion is met with only in conditions associated with an entire absence of digestive juices

FIG. 47.

Collective view of vomited matter. (Eye-piece III., objective 8 A, Reichert.) *a*, muscle-fibres; *b*, white blood-corpuscles; *c*, *c'*, squamous epithelium; *c''*, columnar epithelium; *d*, starch-grains, mostly changed by the action of the digestive juices; *e*, fat-globules; *f*, sarcinæ ventriculi; *g*, yeast-fungi; *h*, forms resembling the comma-bacillus found by the author once in the vomit of intestinal obstruction; *i*, various micro-organisms, such as bacilli and micrococci; *k*, fat-needles, between them connective-tissue derived from the food; *l*, vegetable cells. (v. JAKSCH.)

from the stomach—*i. e.*, in cases of atrophic cirrhosis of the stomach (anadeny of Ewald). This condition is not to be confounded with

[1] Brieger, Untersuchungen über Ptomaine, Hirschwald, Berlin, 1886.

the regurgitation of undigested food, mixed with mucus and saliva, which is seen in cases of stricture of the œsophagus or of the cardiac orifice of the stomach. While at the outset of the latter disease the regurgitation of food occurs immediately, or at least very soon, after a meal, it may take place between meals in the later stages of the disease when dilatation has occurred. The recognition of the origin of the material brought up may then be exceedingly difficult. In such cases an examination should be made for biliary coloring-matter, which, if present, will, of course, immediately exclude the œsophagus as the source of the material ejected. Unfortunately, however, the reverse does not hold good. Small amounts of undigested meat are of no significance. The vomiting of well-digested food is observed in some of the neuroses of the stomach, and also in certain cases of acute and subacute gastritis, ulcer of the stomach, and chronic gastritis in its early stages. The vomiting referable to cerebral and spinal diseases also belongs to this category. In this connection it is very important to inquire into the existence of nausea previous to the vomiting, for, as is well known, considerable amounts of saliva and mucus may be swallowed if much nausea has existed, the result being that the process of digestion is arrested before the occurrence of vomiting. In such an event it would be erroneous to conclude that, because the material ingested has not reached that stage of digestion which would be expected at the time of the vomiting, the stomach is incapable of properly performing its functions.

Mucus.—The constant presence of large amounts of mucus in the gastric contents obtained with the stomach-tube is almost pathognomonic of the mucous form of gastritis, while its presence in vomited matter may be referable to pre-existing nausea. In cases of pharyngitis moderate amounts of mucus are frequently found. The vomiting of pure mucus, according to Boas, is always pathognomonic of the absence of dilatation of the stomach, a statement founded on reason, as it is altogether unlikely that no particles of food should be brought up at the same time.

Under the term *gastrosuccorrhœa mucosa* Dauber[1] has described a condition in which large amounts of mucus are secreted by the non-digesting organ, in the absence of symptoms pointing to a gastritis. I have observed a similar case occurring in a neurasthenic patient, in which enormous quantities of mucus could at times be obtained from the fasting organ, but never during the process of digestion. A mild degree of hyperchlorhydria existed at the same time, as well as enteritis mucosa and rhinitis mucosa. The motor power was practically normal.

Mucus is readily recognized on simple inspection by its glossy

[1] Dauber, "Ueber kontinuirliche Magen-Schleimsecretion," Arch. f. Verdauungskrank., vol. ii. p. 167.

appearance. Chemically, it is distinguished by its behavior toward acetic acid (see Urine).

Saliva.—The vomiting of pure saliva in the morning upon rising is a fairly common symptom of chronic pharyngitis, which in turn frequently carries in its train a chronic gastritis; it constitutes the so-called *vomitus matutinus*. Saliva, like mucus, is, of course, always present in the gastric contents in small amounts. Larger amounts are usually referable to an increased secretion owing to the existence of nausea. Chemically, saliva is best recognized by testing for the presence of the sulphocyanides (see Saliva, page 199).

Bile.—Bile is rarely observed in the gastric contents brought up by the stomach-tube, but is frequently seen in vomited matter, of which it may be said to be a constant constituent whenever the vomiting has been very intense or frequently repeated. Its presence in the former case should always excite suspicion of the existence of stenosis of the descending or horizontal portion of the duodenum or the beginning of the jejunum. This diagnosis becomes the more probable the more constant its presence.

Pancreatic Juice.—Mixed with the bile there is probably always present some pancreatic juice, and it has even been suggested that the constant absence of this constituent, in the presence of bile, is strongly suggestive of pancreatic disease or of obstruction of the pancreatic duct (the ductus Wirsungianus).

Blood.—The presence of unaltered blood in the gastric contents is usually recognized without difficulty. As marked changes in color, varying from a deep red to a coffee or chocolate brown, may occur, however, when free acids are present, it is at times necessary to resort to a more detailed examination. In order to recognize mere traces when the macroscopical and even the microscopical examination do not point to the presence of blood, the method of Müller and Weber or that of Donogany should be employed. Kuttner claims that he was thus able to demonstrate the presence of blood in numerous cases of chlorosis in which other tests furnished negative results. I have been less successful in the disease in question, but admit that in cases of carcinoma and ulcer of the stomach it is with this method often possible to find traces of blood which would otherwise have remained unnoticed.

Method of Müller and Weber.—The gastric contents are treated with a few cubic centimeters of strong acetic acid and extracted with ether. Should the ether not separate in a clear layer after a few minutes, a few drops of alcohol are added. If the ether then remains colorless, no blood-pigment is present, while a brownish-red color indicates the presence of acetate of hæmatin. As a similar but yellowish-brown and much less intense discoloration of the ether may be produced by other pigments, such as biliary coloring-matter, it is well, in doubtful cases, to test the ethereal extract with

tincture of guaiacum. A positive result indicates the presence of blood coloring-matter. The same may be said if, upon spectroscopic examination of the ethereal extract, an absorption-band is discovered at the junction of the red and yellow.

Donogany's Method.—A small amount of the suspected material is extracted with a 20 per cent. solution of sodium hydrate and filtered. A drop of the filtrate is then mixed on a slide with a drop of pyridin and covered with a cover-glass, when, in the presence of blood, orange-red crystals of hæmochromogen will separate out on standing for a few hours. On spectroscopic examination these crystals will show the characteristic band of absorption between the yellow and the green.

Hemorrhage from the stomach, *hæmatemesis*, may be observed in the most diverse conditions. It is either dependent upon a primary disease of the organ, such as ulcer and carcinoma, or it occurs secondarily to disease of other organs, leading to a hyperæmic condition of the gastric mucosa, such as the various forms of cardiac, renal, and hepatic disease, in connection with menstrual abnormalities, etc. In melæna, purpura hæmorrhagica, pernicious anæmia, etc., the cause of the hemorrhage cannot always be determined. It appears to be certain, however, that nervous influences may also take part in the causation of gastric hemorrhage.

Pus.—The occurrence of pus in vomited matter, referable to disease of the stomach itself, is uncommon. It is seen practically only in cases of phlegmonous and diphtheritic gastritis, and, as Strauss[1] has pointed out, in carcinoma affecting the smaller curvature and the region of the fundus. In such cases it is not uncommon to obtain as much as one-half to two tablespoonfuls of a mucopurulent fluid from the non-digesting organ. As the motor function in this form of carcinoma is often unimpaired, the symptom may be of considerable value in diagnosis. The presence of larger quantities usually indicates perforation into the stomach of an accumulation of pus from a neighboring organ. An abscess of the liver, a suppurative pancreatitis, an abscess of the colon, or a subphrenic abscess may thus prove to be its primary source. When present in considerable amount pus is, of course, readily detected with the naked eye; if any doubt should arise, a microscopical examination will determine the question.

Stercoraceous Material.—Very important from a clinical standpoint is the vomiting of stercoraceous matter which is notably observed in cases of ileus. Usually this is recognized without difficulty by its odor, which is referable to the presence of skatol. If any doubt should arise, it is only necessary to distil the vomited matter after the addition of a little phosphoric acid, and to test for the presence of phenol, indol, and skatol in the distillate, as

[1] H. Strauss, "Ueber Eiter im Magen," Berlin. klin. Woch., 1899, p. 870.

described in the chapter on Feces (see page 279). When chiefly derived from the small intestine, the vomited matter, according to v. Jaksch, will contain bile-acids and bile-pigment together with an abundance of fat, which may be detected by chemical or microscopical examination. The reaction is usually alkaline or feebly acid.

I have had occasion to examine the vomited matter of a patient in whom an almost complete obstruction existed immediately above the ileo-cæcal valve; the color of the material was a golden yellow, the reaction neutral; no bile-pigment or biliary acids were found, while hydrobilirubin was present.

Parasites.—Of parasites, ascarides, segments of tæniæ, trichinæ, Anchylostoma duodenale, and Oxyuris vermicularis are, at times, encountered. Trichomonades has been described in the stomach contents of patients with carcinoma, by Hensen, Strübe, Zabel, Ullmann, and Cohnheim. The latter also refers to the occasional presence of amœbæ and of megastoma, and is inclined to attach some diagnostic importance to the presence of such infusoria. He points out that the organisms in question will only find conditions favorable to their growth and development in the carcinomatous, non-insufficient organ; while in non-malignant affections, as also in the malignant forms associated with insufficiency, the absence of an alkaline reaction and of irregularities in the surface of the mucosa (as in atrophy) prevent their active growth. Cohnheim regards their presence as important in the diagnosis of non-pyloric carcinoma, previous to the development of palpable masses, as the presence of lactic acid in the diagnosis of pyloric neoplasm.

The examination for the presence of infusoria should be made at once after withdrawing the material from the stomach. The stomach contents should not be diluted.[1] For a description of these parasites, see the chapter on the Feces.

Odor.—The odor of normal gastric juice is peculiar, suggesting the presence of an acid, which can be sharply distinguished from acetic or butyric acid. If blood is present in large amount, the vomitus emits an odor which is perfectly characteristic. A feculent odor is met with in cases of enterostenosis or in the presence of an abnormal communication between the stomach and the small or large intestine. A putrid odor may be observed in cases of ulcerative carcinoma, pyloric stenosis referable to ulcer, simple carcinoma of the stomach, muscular hypertrophy of the pylorus, stenosis due to inflammatory adhesions, etc. In cases of phosphorus poisoning the vomited matter emits an odor of garlic; the odor observed in uræmic conditions is referable to ammonia; a carbolic acid odor is met with in cases of poisoning with this substance.

[1] G. Strübe, "Trichomonas hominis bei Carcinoma ventriculi," Berlin. klin. Woch., 1898, p. 708. P. Cohnheim, Deutsch. med. Woch., 1903, vol. xxix. p. 206.

MICROSCOPICAL EXAMINATION OF THE GASTRIC CONTENTS.

In the gastric juice obtained from the non-digesting stomach the various morphological constituents of mucus and saliva, which have been described elsewhere, are found. Microscopical particles of food, such as elastic tissue-fibres, starch-granules, fat-droplets, fatty acid crystals, vegetable- and muscle-fibres, are, furthermore, quite constantly seen. Leucocytes and isolated nuclei also are observed; the latter are set free by the action of the gastric juice upon the mucous corpuscles and epithelial cells.

If gastric juice is allowed to stand, small tapioca-like bodies will collect at the bottom of the vessel, which upon microscopical examination will be seen to contain numerous snail-shell-like formations, occurring either singly or collected in groups. These probably consist of altered mucin, as they can be produced artificially by adding a sufficient amount of dilute hydrochloric acid to saliva. According to Boas, they are of no diagnostic significance.

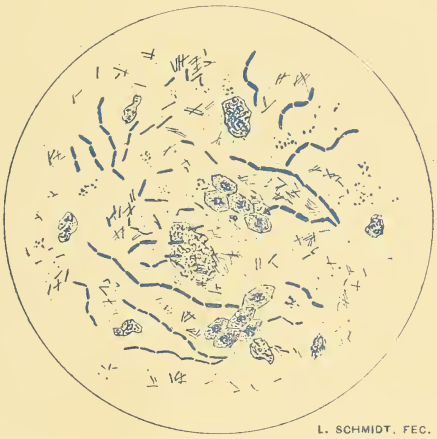
Epithelial cells, fragments of the epithelial lining of the ducts of glands, as well as goblet-cells, are not infrequently met with in the juice obtained from the non-digesting organ. In addition, various micro-organisms, such as the Leptothrix buccalis, Bacillus subtilis, saccharomyces, micrococci (often arranged in the form of tetrahedra), Clostridium butyricum, etc., may be encountered.

Among the bacteria which may be found in the gastric contents under pathological conditions the bacillus described by Boas and Oppler[1] is undoubtedly the most important, and has of late attracted much attention. It appears to be present quite constantly in carcinoma, and is almost always absent in other diseases of the stomach. It is thought that the formation of lactic acid, which is likewise so constantly observed in carcinoma, is largely and perhaps solely referable to its presence. The organism in question (Plate XIV.) is non-motile, and essentially characterized by its great length and by the fact that the individual bacilli are frequently seen joined end to end, forming long threads and zigzag lines which are very characteristic. Often the entire field of vision is filled with dense conglomerations. Cultivation-experiments have thus far not been successful. The organism is readily stained with the usual anilin dyes.

Tubercle bacilli may be found in vomited matter in cases of phthisis, where the sputa have been swallowed. Tubercular ulceration of the stomach is exceedingly rare. Simmonds reports that in 2000 autopsies of tubercular individuals the condition was noted only eight times.

[1] Oppler-Boas, "Zur Kenntniss des Mageninhalts bei Carcinoma ventriculi," Deutsch. med. Woch., 1895, No. 5. Kauffmann, "Ueber einen neuen Milchsäurebacillus," etc., Wien. klin. Woch., 1895, No. 8. Schlesinger u. Kauffmann, Wien. klin. Rundschau, 1895, No. 15.

PLATE XIV.



The Boas-Oppler Bacillus, Stained with Methylene Blue. From a Case of Carcinoma of the Large Curvature of the Stomach. (Personal Observation.)

Sarcinæ (Fig. 47) occur in the form of peculiar colonies of cocci, arranged in squares or tetrahedra, strongly resembling cotton-bales. Not infrequently they are encountered under normal conditions, but only in small numbers. In pathological conditions, on the other hand, a drop of the gastric contents may constitute an almost pure culture. A case is even on record in which the pylorus had become entirely occluded by an inspissated mass of these organisms. Whenever present the existence of certain fermentative processes may be inferred.

It is curious to note that in advanced cases of carcinoma of the stomach sarcinæ are practically never seen, although the conditions are apparently most favorable for their development. Oppler[1] was unable to find them twenty-four hours after their introduction in

FIG. 48.

Cancer-cells from the gastric contents. (EWALD.)

large numbers and in pure culture. In cases of carcinoma of the curvatures and the walls, as also in advanced pyloric carcinoma, sarcinæ were never found, while they may be present in incipient cases of pyloric carcinoma so long as hydrochloric acid is secreted.

In vomited material containing biliary coloring-matter, leucin, tyrosin, and cholesterin are quite commonly observed, and may be recognized by the form of their crystals, as well as by their chemical reactions, which are described elsewhere.

The occurrence of blood and pus in the gastric contents has been considered (see pages 261 and 262).

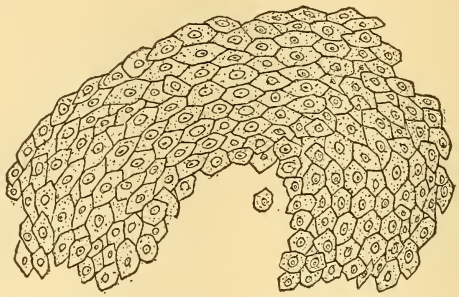
It not infrequently happens that small shreds of mucous membrane are brought away by the stomach-tube, and in cases of chronic gastritis, hyperchlorhydria not dependent upon ulcer, and in some

[1] Oppler, Münch. med. Woch., 1894, No. 29.

of the neuroses, this is indeed not at all uncommon.[1] Boas even suggests that in the neuroses, where fragments of mucous membrane are so readily detached, this may possibly be connected etiologically with the formation of ulcers, and there can be no doubt that the mere action of the abdominal muscles exerted during the process of defecation may be sufficient to detach such fragments. From the microscopical appearance of the particles the diagnosis between a gastric neurosis and one of the various forms of chronic gastritis may frequently be made, and the same may be said to hold good in the differential diagnosis between a true gastritis and a glandular insufficiency referable to passive congestion of the gastric mucosa.

At times *tumor particles* also are found in the gastric contents.[2] In the accompanying illustration (Fig. 48) a specimen obtained from a

FIG. 49.



A fragment of mucous membrane derived from the stomach. (EWALD.)

carcinomatous patient is represented, which is readily distinguished from similar fragments of mucous membrane (Fig. 49).

EXAMINATION OF THE MOTOR POWER OF THE STOMACH.

Under physiological conditions the stomach should contain but few particles of food, or none at all, six hours after the ingestion of Riegel's meal, or one and one-half to one and three-quarters hours after that of Ewald. A delay in the propulsion of the gastric contents may be referable to the existence of a simple atony or to dilatation of the stomach. According to Boas, an atony may usually be diagnosed if, following the exhibition of a supper consisting of bread and butter, cold meat, and a large cupful of tea, the stomach is found empty in the morning, providing, of course, that symptoms exist which point to atony or dilatation. It should be remembered, however, that in cases of acute and subacute gastritis, in the absence of a more

[1] M. Einhorn, Med. Record, June 23, 1894; Berlin. klin. Woch., 1895, No. 20; Arch. f. Verdauungskrankheiten, vol. v. Heft 3.

[2] P. Cohnheim, "D. Bedeutung kleiner Schleimhautstückchen f. d. Diagnostik d. Magenkrankheiten," Arch. f. Verdauungskrankheiten, 1896, vol. i. p. 274.

serious lesion, food may be found in the stomach twenty-four hours after its ingestion. A dilatation may, on the other hand, be diagnosed if the stomach under the same conditions contains a considerable amount of food. In such cases it happens that not only remnants of the test-supper, but remains of meals taken one, two, three, or even more days previously are found. The quantities, moreover, which may be obtained at the time of examination are often surprisingly great, and may amount to sixteen pounds or more. Portel cites the case of the Duc de Chausnes, one of Paris' greatest gourmands, whose stomach could hold 4.5 liters—*i. e.*, 8 pints.

The following methods may be employed for the purpose of testing the motor power of the stomach:

Leube's Method.[1]—Six hours after the ingestion of Riegel's meal the stomach is washed out with about 1000 c.c. of water. In the presence of only slight traces of food the motor power may be regarded as normal. This method is undoubtedly the most convenient for practical purposes.

The Salol Test of Ewald and Sievers.[2]—This test is based upon the observation that salol, a compound ether of salicylic acid, is decomposed into phenol and salicylic acid only in an alkaline medium. As the salicylic acid is eliminated in the urine as salicyluric acid, it is possible to determine the time of the passage of the salol from the stomach into the small intestine.

A capsule containing 1 gramme of salol is given to the patient immediately after his breakfast or dinner, when separate portions of urine, passed one-half, one hour, two hours, and twenty-four hours later, are tested by adding a small amount of a solution of ferric chloride. In the presence of salicyluric acid a violet color results. Under normal conditions a positive reaction is obtained after from forty-five to seventy-five minutes. A further delay may usually be regarded as indicating the existence of motor insufficiency. If no result is obtained after twenty-four hours, a pyloric stenosis undoubtedly exists. Under normal conditions, furthermore, it will be observed that the salol elimination is completed after twenty-four hours, while in cases of dilatation of the stomach a positive reaction may still be obtained after thirty hours. It is thus possible to distinguish between dilatation and descent of the stomach.

The test, while it is convenient and usually yields fair results, is not altogether reliable, as the decomposition of the salol may at times occur in the stomach, owing to the presence of alkaline mucus, or may be delayed in the intestines owing to the existence of acid fermentation, etc.[3]

[1] Leube, Deutsch. Arch. f. klin. Med., vol. xxxiii.
[2] Ewald u. Sievers, Therap. Monats., August, 1887.
[3] Brunner, Deutsch. med. Woch., 1889. Huber, Correspondenzbl. f. schweizer Aerzte, 1890.

EXAMINATION OF THE RESORPTIVE POWER OF THE STOMACH.

To this end, a capsule containing 0.2 gramme of potassium iodide is given to the patient shortly before a meal, and the saliva examined for the presence of potassium iodide at intervals of from two to three minutes[1] (see Saliva, page 202). Under normal conditions a violet color is obtained after from six and one-half to eleven minutes, and a bluish tint after from seven and one-half to fifteen minutes. In pathological conditions a delayed reaction is observed in almost all diseases of the stomach, and is especially marked in cases of dilatation and carcinoma, less so in chronic gastritis, and variable in ulcer.

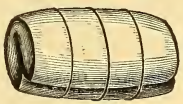
Absolute conclusions, however, cannot be drawn from results thus obtained, as a normal reaction-time has also been observed in cases of dilatation and chronic gastritis.

INDIRECT EXAMINATION OF THE GASTRIC JUICE.

Günzburg's Method.[2]—In those cases in which for any reason the introduction of the stomach-tube is contraindicated or impracticable the following method, suggested by Günzburg, may be employed:

A tablet of 0.2 to 0.3 gramme of potassium iodide is inserted into a piece of the thinnest possible, strongly vulcanized rubber-tubing, measuring about 2.5 cm. in length. The ends are folded as shown

FIG. 50.



A fibrin-potassium-iodide package of Günzburg.

in Fig. 50, and the little package tied with three threads of fibrin hardened in alcohol. Every package should be examined before use, by immersion in warm water for several hours, to determine its tightness, testing for the presence of potassium iodide by means of starch-paper and fuming nitric acid. One of these packages is swallowed by the patient three-quarters to one hour after an Ewald test-breakfast, and the saliva tested for potassium iodide at intervals of fifteen minutes, until a positive result is reached or until six hours have elapsed. It is unnecessary to wait longer than six hours. In the presence of free hydrochloric acid the threads of fibrin are dissolved and the potassium iodide absorbed. Under normal conditions a positive reaction is obtained after from one to one and three-quarters hours, while anachlorhydria undoubtedly exists if no result is obtained within five or six hours. In cases of hypochlorhydria the reaction is delayed for more than two to three hours. Günzburg

[1] Penzoldt, Berlin. klin. Woch., 1892. Faber, Inaug. Diss., Erlangen, 1882.
[2] Sahli, Klinische Untersuchungsmethoden, 1900, p. 399.

further advises that the resorption-test with potassium iodide be also made, and that the reaction-time be deducted from that taken up in the elimination of the iodide contained in the package. Several tests, moreover, should be made in the same case.

I have had occasion to experiment with packages obtained from Germany, and manufactured according to the directions of Günzburg.[1] In most of the packages the threads of fibrin had become brittle and were broken in transit. The results obtained with about twenty intact specimens, however, were entirely satisfactory, and it is to be regretted that the packages cannot be obtained in the American market.

Similar packages have been constructed by Sahli.

Reach has of late made use of barium iodate and the oxyiodate of bismuth for the same purpose, but without enclosing the substance in rubber. As hydrochloric acid only is capable of liberating the iodine from these bodies, they may be employed instead of the Günzburg packages. As a result of his examinations, he concludes that in the presence of hydrochloric acid iodine can thus be demonstrated in the saliva within eighty minutes. He finds, however, that at times the reaction occurs later than might have been supposed from the amount of hydrochloric acid found.

Simon's Method.—Personal researches have led me to believe that a close relation exists between the elimination of indican in the urine and the amount of free hydrochloric acid in the gastric contents.[2] The results reached may be summarized as follows:

1. Euchlorhydria is associated rarely with an increased elimination of indican.

2. In cases of simple neurotic hyperchlorhydria a subnormal or normal amount of indican is the rule.

3. In cases of hyperchlorhydria associated with ulcer an increased indicanuria is observed quite constantly.

4. Anachlorhydria referable to organic lesions of the stomach is associated almost invariably with a highly increased indicanuria.

5. Hysterical anachlorhydria may be associated with the elimination of a normal or increased amount of indican.

6. In cases of hypochlorhydria increased indicanuria is the rule.

Given as premises:

1. That a resorption of decomposing pus is not taking place anywhere within the body, as such a process in itself is capable of causing an increased elimination of indican.

2. That a stenosis of the small intestine or a high degree of gastric atony does not exist.

3. A normal mixed diet, containing no excessive amounts of red meat (see Indicanuria).

[1] Göthe Apotheke, Frankfurt a. M.

[2] C. E. Simon, Am. Jour. Med. Sci., 1895, vol. cx. p. 481.

CHAPTER IV.

THE FECES.

THE feces constitute a mixture of indigestible and undigested particles of food, of unabsorbed secretions of the gastro-intestinal tract, and their decomposition-products together with intestinal mucus, epithelial cells, and bacteria.

EXAMINATION OF NORMAL FECES.

General Characteristics.

Number of Stools.—The number of stools which may be passed in the twenty-four hours is subject to wide variation, even under physiological conditions, but is usually constant for one and the same individual. One or two stools *pro die* may be regarded as normal. Exceptions, however, are frequent. Persons are thus met with who have but one stool every two to four days, and cases are on record in which only one passage occurred every seven to fourteen days, the individuals evidently enjoying perfect health. On the other hand, the number of stools may be increased to three or four under strictly normal conditions. *Hence the importance of accurately ascertaining the habitual number of stools in every individual.* It would thus be manifestly wrong to regard the passage of three stools daily as diarrhœa, or the passage of only one stool in forty-eight hours as constipation, if this number has been habitual throughout life.

Whether or not it is permissible to regard as normal those rare instances in which only one stool occurs every two to six weeks, or even less frequently, is rather doubtful.

Amount.—In those cases in which more than one or two stools occur in twenty-four hours it is well to ascertain the amount actually passed. The normal amount varies between 100 and 200 grammes.[1] This quantity is increased by a diet rich in vegetable and starchy foods, and is diminished by one rich in animal proteids, so that 60 and 270 grammes may be regarded as the extreme limits in health. Such amounts as 500 and 1000 grammes are certainly abnormal.

[1] Voit, Zeit. f. Biol., vol. xxv. p. 264.

Average quantities for various ages are given in the following table, which is taken from Schmidt and Strassburger :[1]

Age.	Diet.	Average amount of feces in twenty-four hours.
Child, 1 month old	Mother's milk	3.3 Grammes.
" 2–3 months old	" "	6.5 "
" 7 " "	Variable	15–56 "
" 9 " "	Cows' milk with additions	59.0 "
" ¾–2 years old	Mixed	77.0 "
" 4 " "	"	101.0 "
" 6 " "	"	134.0 "
" 9 " "	"	117.0 "
" 11 " "	"	138.0 "
Adult	"	131.0 "

Consistence and Form.—The consistence of a stool depends essentially upon the amount of water present, and hence upon the nature of the food ingested, being softer with a purely vegetable diet (80–85 per cent. of water) than with a diet rich in animal proteids (60–65 per cent.). With a mixed diet the amount of water corresponds to about 75 per cent. As a general rule, normal stools exhibit the characteristic cylindrical form and are fairly firm. Mushy stools, however, are also seen quite frequently, and round, scybalous masses, although far more common in constipation, may likewise be observed in health.

Odor.—The repugnant odor of the feces is, to a large extent, due to the presence of indol and skatol ; hydrogen sulphide, methane, and traces of phosphin may add still further to their disagreeable odor.

Color.—The color of the feces varies, according to the nature of the food ingested, from a light to almost a blackish brown, a firm stool being in general darker than a thin stool. A stool that has remained exposed to the air is also somewhat darker upon its outer surface than in its interior, owing to processes of oxidation. In nursing-infants, in consequence of the exclusive ingestion of milk, the color is light yellow.

Under normal conditions the color is never due to native biliary coloring-matter, but is largely dependent upon the presence of urobilin (see page 283). It is, furthermore, influenced by the nature of the food, chlorophyll tending to produce a greenish color, starches a yellowish tinge. If much blood is present in the food, the feces may be almost black, owing to the formation of hæmatin. Huckleberries and red wine likewise produce a blackish color, chocolate and cocoa a gray ; preparations of iron, manganese, and bismuth color the feces dark brown or black, owing to the formation of sulphides of these metals ; the green color of calomel stools was formerly

[1] A. Schmidt and J. Strassburger, Die Faeces d. Menschen, Berlin, 1961, A. Hirschwald.

supposed to be due to the formation of a sulphide, but is more likely caused by the presence of biliverdin. Santonin, rheum, and senna produce a yellow color.

Macroscopical Constituents.

Alimentary Detritus.—Upon further examination of the feces it is possible to find visible to the naked eye undigested particles of food, which are partly indigestible and partly digestible, such as stones of cherries, grape-seeds, woody vegetable fibre, the skins of berries, large pieces of connective tissue, undigested pieces of apple, pear, potato, grains of corn, etc. Such undigested food is found in abundance when insufficiently masticated or taken in excessive amounts.

Flakes of caseïn, recognizable with the naked eye, are also frequently seen. Care should be taken not to confound these with particles of stool composed of fatty acid crystals. This mistake is often made, and can readily be avoided by a microscopical or chemical examination (see page 295).

Foreign Bodies.—In children, the insane, in cases of hysteria, and even in people who are apparently possessed of their normal senses, the physician must be prepared to find at times all kinds of foreign bodies, such as pins, coins, buttons, false teeth, tooth-plates with ragged edges, and even dirk-knives, all of which have been known to pass through the alimentary canal with perfect safety. It must not be forgotten, however, that in certain cases of hysteria bodies may be shown by patients which they claim have passed by the rectum, but which have been wilfully added to the stools, such as snakes, frogs, etc.

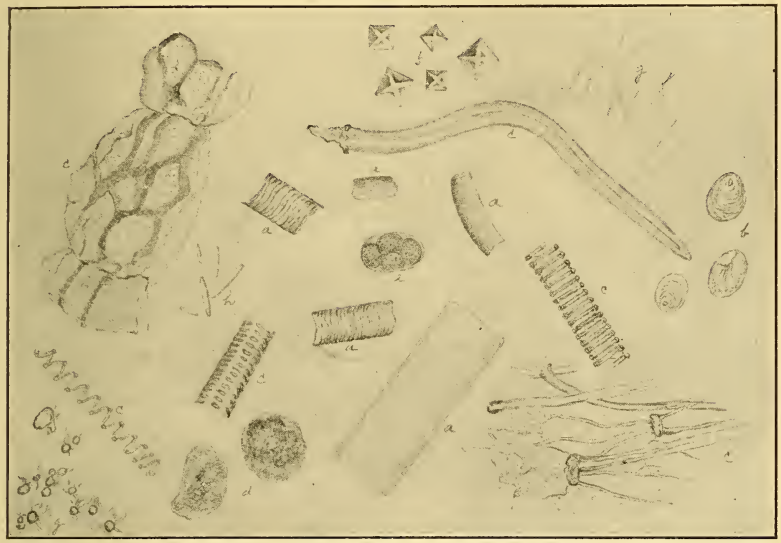
Microscopical Constituents.

Constituents derived from Food.—Microscopically, indigestible and undigested constituents of food may be seen (Fig. 51), such as the framework of vegetable material, sometimes still containing starch-granules or remnants of chlorophyll; muscle-fibres, usually colored yellow and more or less altered in structure. Elastic-tissue fibres are readily recognized by their double contour and bold outlines. Connective-tissue fibres of the white fibrous variety can also generally be distinguished; when present in large quantities, however, they are usually indicative of some digestive derangement, unless they are observed following the ingestion of a meal particularly rich in meat. Flakes of caseïn also are seen frequently.

Muscle-fibres are found in every stool whenever meat has been eaten. Under normal conditions, however, they are not numerous, unless particularly large quantities have been ingested. Their ap-

pearance under the microscope may vary considerably. On the one hand, fibres are met with which still retain their characteristic

FIG. 51.



Collective view of the feces: *a*, muscle-fibres; *b*, starch-granules; *c*, vegetable material; *d*, potato-cells; *e*, egg of Uncinaria duodenalis; *f*, calcium oxalate crystals; *g*, fatty acid crystals; *h*, Charcot-Leyden crystals.

features; others are split up either partially or entirely into the well-known disks; but more common than both are more or less roundish, yellow, apparently homogeneous fragments, which at first sight do not resemble muscle-fibres in the least. Upon closer investigation, however, their true nature will become apparent. It will then be seen that two of the sides in some portions at least are more or less parallel, and if the specimen is examined with an oil-immersion lens some traces of cross-striation can probably always be discovered.

Isolated starch-granules are scarcely ever found under normal conditions, excepting in young children who have been fed with much starchy material. Starch-granules enclosed in vegetable cells are likewise not found as a general rule, but are more common than the isolated granules. The presence of either in large numbers is usually indicative of the existence of some pathological condition affecting the gastro-intestinal tract. Their presence is easily recognized by treating microscopical preparations with a solution of iodo-potassic iodide (Lugol's solution), when the granules or fragments will assume a blue color.

The presence of fat in the feces is quite constant, even in health. It may occur in the form of needle-like crystals, as fat-droplets, or

as polygonal masses which are highly refractive and often colored yellow or a yellowish red. Their true nature is easily recognized by adding a drop of concentrated sulphuric acid and heating, when they are transformed into the characteristic fat-droplets.

Morphological Elements derived from the Alimentary Canal.—1. Epithelial cells. Well-preserved cylindrical or goblet cells are only exceptionally found in the feces, while transition-forms from the normal cells to mere spindles, in which a nucleus can no longer be recognized, are observed quite constantly. These degenerative changes, according to Nothnagel,[1] are the result of an abstraction of water from the cells, which may alter their appearance to an extent that only the experienced eye is capable of recognizing their true character. Pavement epithelial cells, when present, are derived from the anal orifice.

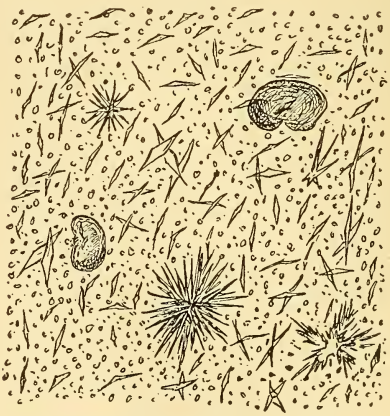
2. Leucocytes are almost always absent in normal stools or present only in very small numbers.

3. Red blood-corpuscles in very small numbers are occasionally observed under apparently normal conditions, but are then of no significance.

4. In every stool a large number of structureless granules may be seen, lying either by themselves or collected into heaps; they are designated as detritus.

Crystals.—Needle-like crystals of free fatty acids, and the cal-

FIG. 52.



Fatty crystals obtained from the feces.

cium and magnesium salts of the higher members of this group, occurring either singly or arranged in sheaves, may be found in every stool (Fig. 52). They are of no significance unless present

[1] Nothnagel, Beiträge z. Physiol. u. Pathol. d. Darmes, Hirschwald, Berlin, 1884, and Specielle Pathol. u. Therap., Hölder, Wien, 1895, vol. xvii. Pt. 1.

in large numbers. Nothnagel[1] speaks of the frequent occurrence of certain calcium salts (of fatty acids, as he believes) in normal as well as pathological stools. He states that they are almost always bile-stained, and occur in irregular, sometimes elliptical, oval, or circular masses, in which a crystalline structure cannot be distinguished. They are apparently of no importance. Quite common, also, are crystals of neutral calcium phosphate and ammonio-magnesium phosphate, the former occurring in the form of more or less well-defined wedge-shaped crystals collected into rosettes, the latter presenting the well-known coffin-shape when the stool is mushy, while in firm stools irregular fragments mostly are found. At one time the ammonio-magnesium phosphate crystals were supposed to be characteristic of typhoid stools, but it is now known that they occur in normal feces, as well as under the most varied pathological conditions. Their presence is of no diagnostic significance. It is important to note that the neutral phosphates are never stained by bile-pigment, and the triple phosphates only in rare instances. Both are easily soluble in acetic acid. Crystals of calcium oxalate may be found in abundance following the ingestion of certain vegetables, such as sorrel and spinach. They are usually found imbedded in the vegetable débris. They are readily recognized by their characteristic envelope-form, their insolubility in acetic acid, and their solubility in hydrochloric acid. Not infrequently they are bile-stained.

Calcium lactate is frequently seen in the stools of children receiving a milk-diet; they occur in the form of sheaves composed of radiating needles. Calcium carbonate is rarely observed, but occasionally occurs in the form of amorphous granules or dumb-bell-shaped crystals. Calcium sulphate crystals are likewise rare, but may be produced artificially by the addition of sulphuric acid, when beautiful needles and platelets may be observed. Cholesterin, while always present in solution, is rarely observed in crystalline form (Fig. 53). I have found it only twice in several hundred examinations. Hæmatoidin crystals are never found in normal stools. Charcot-Leyden crystals may be found under pathological conditions; according to my experience, they are never seen in normal stools.

Parasites.—The parasites which occur in normal feces may be divided into vegetable and animal parasites.

Vegetable Parasites.—These are always present in enormous numbers. What relation they bear to the process of digestion is still an open question. The idea held by Pasteur and many others, that animal life cannot go on in the absence of bacteria from the digestive tract has been disproved by Nuttall and Thierfelder.[2] A

[1] Loc. cit.

[2] Nuttall u. Thierfelder, "Thierisches Leben ohne Bakterien im Darm," Zeit. f. physiol. Chem., 1896, vol. xxi. p. 109, and 1897, vol. xxii. p. 62.

guinea-pig, removed by Cæsarean section from the uterus of the mother-animal, under antiseptic precautions, was placed in a sterilized glass cage and nourished for a week with sterilized food. The air which the animal breathed was likewise sterilized. During this week the animal consumed about 330 c.c. of milk and appeared to be normal in every respect. At the expiration of the week it was killed, when a microscopical examination of the intestinal contents revealed the absence of bacteria. Culture-experiments also were negative.

Macfadyen, Nencki, and Sieber[1] likewise found that their now so often quoted fistula patient continued in good health, and even gained flesh, although the entire large intestine, in which bacterial activity is always greatest, was isolated for a period of many weeks.

Fungi.—Fungi, with the exception, perhaps, of the Oïdium albicans, which has at times been observed, are rarely found in the feces.

Schizomycetes.—Saccharomyces cerevisiæ is one of the normal constituents of the feces, and is found in its characteristic forms, three or four buds, however, being but ordinarily observed. Owing to the glycogen present in their substance, they assume a mahogany color when treated with a solution of iodo-potassic iodide. They should not be confounded with a class of bacteria which closely resemble the saccharomyces in general appearance, but are colored blue when treated in the same manner (see below).

Bacteria.—The bacteria are the micro-organisms *κατ'ἐξοχήν* which are found in the feces. Their number is truly enormous. Sucksdorff thus found in his own person that on an average 53,124,000,000 were eliminated in the twenty-four hours under normal conditions. About 97 per cent. of these are directly derived from the ingested food, and the remaining 3 per cent. from swallowed saliva. If we recall the strongly bactericidal power of the gastric juice, such an observation must at first sight appear most surprising. It should be remembered, however, that the spores of the bacteria are far less susceptible to the action of hydrochloric acid, and that large amounts of the ingesta are carried into the small intestine at a time already, when hydrochloric acid has not as yet appeared in the free state.

On the whole, the bacteriological flora of the intestinal contents is fairly constant, but, as in the other cavities and channels of the body where bacteria are invariably met with, transient guests are also not uncommon. The majority of the bacteria which are here encountered are, as a general rule, harmless; but it is important to note that under suitable conditions a number of these may develop pathogenic properties. Broadly speaking, the bacteria which may

[1] Macfadyen, Nencki, u. Sieber, "Untersuchungen über die chemischen Vorgänge im menschlichen Dünndarm," Arch. f. exper. Path. u. Pharmakol., 1891, vol. xxviii. p. 311.

be found normally in the feces can be divided into two classes. Those belonging to the first order are stained a yellow or a yellowish brown with iodo-potassic iodide, while those belonging to the second class are colored blue or violet by the same reagent. To the former belong the Bacterium termo, the Bacillus subtilis, and a large number of micrococci; into a description of these it is not necessary to enter at this place.[1] Under the second heading v. Jaksch[2] describes the following forms:

1. Micrococci occurring in the zoöglœa stage, which are colored a violet red.

2. Short, thin rods, tapering slightly at both ends, and in their microscopical appearance much resembling the bacillus of the septicæmia of mice; sometimes they contain one or two little bodies, which are not stained by the reagent.

3. Short or long rods, which resemble the Leptothrix buccalis in their behavior toward iodo-potassic iodide.

4. Bacilli resembling the Bacillus subtilis.

5. Bacillus butyricus. This micro-organism, according to Brieger, is the cause of butyric acid fermentation. It occurs in the form of broad rods with rounded extremities, but may also be elliptical or spindle-shaped. With Lugol's solution it is colored blue or violet, either entirely or only in its central portion.

6. Large round forms, characterized, when unstained, by a pale lustre, and which very much resemble yeast-cells (see above).

7. Micrococci, which assume a reddish, but not very pronounced tint.

It should be mentioned that this second class of micro-organisms is not so largely represented in the feces as the first.

To speak more specifically, the following bacteria have thus far been isolated from the feces: the Bacillus coli communis, Bacterium lactis aërogenes, Bacillus subtilis, Proteus vulgaris, Bacillus putrificus coli, Bacillus liquefaciens ilei, Bacterium ilei, Bacterium ovale ilei, Bacillus gracilis ilei, the veil bacillus of Escherich, Bacillus butyricus, Bacillus Uptadel; Streptococcus coli gracilis, Streptococcus coli brevis, Streptococcus liquefaciens ilei, Streptococcus pyogenes duodenalis, Staphylococcus liquefaciens albus, Staphylococcus liquefaciens flavus, Micrococcus ovalis, the porcelain-coccus of Escherich, tetradenococcus. In addition, various other bacteria have been found, but have not as yet been obtained in pure culture. This is true more particularly of certain forms of spirillum.

The specific pathogenic bacteria which may be found in the feces, as well as those above mentioned, which may at times develop pathogenic properties, will be described in detail later on.

Animal parasites are probably never present under strictly normal conditions.

[1] Flügge, Die Microorganismen.
[2] v. Jaksch, Klinische Diagnostik, 1896.

Chemistry of Normal Feces.

Reaction.—The reaction of the feces is usually alkaline, sometimes neutral, rarely acid, the alkalinity being due to ammoniacal fermentation, the acidity to lactic and butyric acid fermentation, taking place in the intestines. In infants the stools are normally acid.

General Composition.—The following table, taken from Gautier, will give an idea of the composition of fresh feces, calculated for 1000 parts by weight:

	Adult man.	Suckling.
Water	733.00	851.3
Solids	267.00	148.7
Total organic material	208.75	137.1[1]
Total mineral material	10.95[2]	13.6
Alimentary residue	83.00	

The organic material yielded:

Aqueous extract	53.40	53.50
Alcoholic extract	41.65	8.20
Ethereal extract	30.70	17.60[3]

In addition, there are gases, which vary in quantity according to the nature of the food ingested, such articles as beans, heavy bread, potatoes, etc., increasing the amount very considerably.

	Milk diet. Per cent.	Meat diet. Per cent.	Vegetable diet. Per cent.
Carbon dioxide	9–16	8–13	21–34
Hydrogen	43–54	0.7–3	1.5–4
Marsh gas	0.09	26–37	44–55
Nitrogen	36–38	45–64	10–19

Of these gases, carbon dioxide is partly referable to alcoholic and butyric acid fermentation, and partly to albuminous putrefaction, taking place in the intestines. Marsh gas is formed during the fermentation of cellulose, while the nitrogen has partly been swallowed and is partly referable to albuminous putrefaction. A portion also is probably derived from the blood, and it may be mentioned in this connection that the enormous quantities of carbon dioxide so often discharged in cases of hysteria are undoubtedly referable to this source, the gas passing from the blood through the gastro-intestinal mucous membrane into the stomach and intestines.

In order to give a general idea of the chemical constituents of the feces these may be divided into:

1. Food material which could be assimilated, but which was taken in excess, such as starches, fats, and a small amount of non-assimilated albuminous material.

2. Indigestible substances, such as chlorophyll, gums, pectic

[1] Including 54 parts of mucin, epithelium, and calcareous salts.
[2] Not comprising earthy phosphates.
[3] Of this, 3.2 is cholesterin.

products, resins, various coloring-matters, nucleins, chitin, and insoluble salts, viz., silicates, sulphates, earthy phosphates, ammonio-magnesium phosphate, etc.

3. Products derived from the digestive canal, as mucus, partly transformed biliary acids, dyslysin, cholesterin, lecithin.

4. Substances in process of absorption, as emulsified fats, fatty acids, leucin, and biliary acids.

5. Products of decomposition, referable to microbic activity, such as fatty acids, comprising the entire series from acetic to palmitic acid, the latter being especially abundant ; lactic acid, phenol, cresol, indol, skatol, excretin, leucin and tyrosin ; phenol-propionic, phenyl-acetic, hydroparacumaric, and parahydroxyl-phenyl-acetic acids ; ammonium carbonate, and ammonium sulphide.

6. Products of metabolism eliminated through the intestines : urea, uric acid, and xanthin-bases.

7. Pigments : stercobilin, hæmatin, hydrobilirubin, coloring-matter derived from the blood, and, in abnormal conditions, bile-pigments.

8. Water.

9. Gases, as carbon dioxide, marsh gas, hydrogen, and nitrogen.

The study of these substances as a whole, as well as in detail, is of great importance, not only from the standpoint of the physiologist, but also from that of the clinician, giving, together with a careful urinary analysis, the clearest idea of the metabolic processes taking place in the body.

The chemical study of the feces, however, has so far received but little attention, and data of practical importance have scarcely been obtained from the work accomplished. The field is nevertheless an important one.

It is impossible to give here a detailed description of the various chemical constituents which have been mentioned. Only the most important ones, and those especially interesting from a physiological and pathological standpoint, will be considered.

Phenol, Indol, and Skatol.—Phenol, indol, and skatol are formed during the process of albuminous putrefaction, and are constant constituents of the feces. A small portion is resorbed from the intestinal canal, and appears in the urine in combination with sulphuric acid and to a slight extent also with glucuronic acid. Previously, however, the indol and skatol are oxidized to indoxyl and skatoxyl, respectively (see Urine).

To demonstrate the presence of phenol, indol, and skatol in the feces, we may proceed as follows :

The feces are diluted with water, acidified with phosphoric acid, and distilled. The volatile fatty acids present, together with phenol, indol, and skatol, pass over. The distillate is then neutralized with sodium carbonate and again distilled. During this process phenol,

indol, and skatol pass over, the fatty acids remaining behind as sodium salts. In order to separate the phenol from indol and skatol, the distillate is alkalinized with potassium hydrate and again distilled. The phenol now remains behind, and may be obtained in pure form by distilling with sulphuric acid; in this final distillate its presence may be demonstrated by the following reactions:

1. With ferric chloride phenol yields an amethyst-blue color.

2. With bromine-water a crystalline precipitate of tribromophenol is obtained.

3. Treated with Millon's reagent—*i. e.*, the acid mercuric nitrate—a red color develops.

Indol and skatol pass over after treating the above mixture of the three with potassium hydrate and distilling. These two bodies may then be separated from each other by taking advantage of their different degrees of solubility in water.[1]

Indol forms small plates, melting at 52° C., which are easily soluble in hot water, alcohol, and ether; its odor is feculent.

Reactions of indol: 1. When treated with nitric acid and a little sodium nitrite a crystalline red precipitate of the nitrate of nitroso-indol is obtained. 2. A small piece of pine wood moistened with an alcoholic solution of indol acidified with hydrochloric acid is colored a cherry red.

Skatol crystallizes in plates which melt at 95° C. They are soluble with more difficulty in water than indol, and emit a feculent odor.

Reactions of skatol: 1. With nitric acid and sodium nitrite only a milky cloudiness results. 2. Pure skatol does not color pine wood moistened with hydrochloric acid; but if a bit of the wood is saturated with a dilute alcoholic solution of skatol and then immersed in strong hydrochloric acid, it assumes a cherry-red and later a bluish-violet color. 3. With nitric acid of a specific gravity of 1.2 it gives a marked xanthoproteic reaction on boiling—*i. e.*, a yellow color which turns to orange upon the addition of an excess of ammonia.

The determination of cresol in the presence of phenol, together with which it is obtained, is, when only small quantities of these substances are present, a difficult matter. They may be separated from each other by transforming both into their sulpho-acids; the barium salt of para-sulpho-phenol is practically insoluble in barium hydrate.

Fatty Acids.—The chemical composition of fatty acids present in the feces, as well as the relation existing between them, is shown in the table below. The formula C_nH_{2n+1}, COOH, or $C_nH_{2n}O_2$ expresses their general structure.

[1] C. E. Simon, Physiological Chemistry, Lea Bros. & Co., 1901.

Formic acid	H.COOH	= C H_2 O_2
Acetic acid	CH_3.COOH	= C_2 H_4 O_2
Propionic acid	CH_3.CH_2.COOH	= C_3 H_6 O_2
Butyric acid	CH_3.$(CH_2)_2$.COOH	= C_4 H_8 O_2
Isobutyric acid	$(CH_3)_2$.CH.COOH	= C_4 H_8 O_2
Valerianic acid	CH_3.$(CH_2)_3$.COOH	= C_5 $H_{10}O_2$
Caproic acid	CH_3.$(CH_2)_4$.COOH	= C_6 $H_{12}O_2$
Capric acid	CH_3.$(CH_2)_8$.COOH	= $C_{10}H_{20}O_2$
Palmitic acid	CH_3.$(CH_2)_{14}$.COOH	= $C_{16}H_{32}O_2$
Stearic acid	CH_3.$(CH_2)_{16}$.COOH	= $C_{18}H_{36}O_2$

These acids are derived partly from fats, partly from carbohydrates, and to some extent also from proteids.

Separation of the Fatty Acids from the Feces.—If the distillate, neutralized with sodium carbonate, referred to in the above method, is again distilled, the sodium salts of the fatty acids remain behind:

$$2C_{15}H_{31}.COOH + Na_2CO_3 = 2C_{15}H_{31}.COONa + H_2O + CO_2.$$

The solution is then evaporated to dryness on a water-bath, the residue extracted with alcohol, the alcohol evaporated, and the final residue dissolved in water. This solution may now be further examined. In order to separate the different fatty acids from each other, it is best, if the quantity is sufficiently large, to transform them into their silver or barium salts, and to separate these by their varying degrees of solubility in water or by fractional distillation.

General properties of the fatty acids: they are all monobasic, and soluble in water, alcohol, and ether. Their alkaline salts are readily soluble in water and alcohol, but insoluble in ether. The silver salts are dissolved with difficulty.

1. *Formic acid* is a colorless liquid, of a penetrating odor, boiling at 100° C. A concentrated solution of its alkaline salts is precipitated by silver nitrate; the silver salt becomes black on standing, and reduction takes place at once upon the application of heat. Treated with ferric chloride in neutral solution it yields a blood-red color, which disappears on boiling, while a rust-colored precipitate is formed at the same time.

2. *Acetic acid* is a liquid of a pungent odor, which boils at 119° C. After neutralization a blood-red color is obtained on the addition of ferric chloride. Neutral solutions of its salts with the alkalies yield a precipitate with silver nitrate, which is soluble in hot water without reduction taking place.

3. *Propionic acid* is an oily fluid, boiling at 117° C. With ferric chloride no red color results; with silver nitrate it behaves like formic acid.

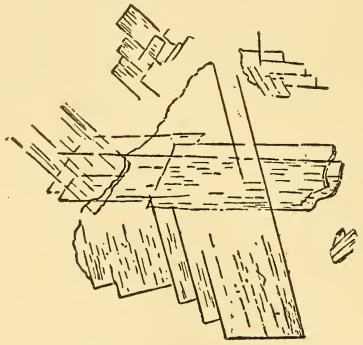
4. *Butyric acid* is an oily liquid, boiling at 137° C.; its odor is similar to that of rancid butter. When treated with an acid, its salts give off the characteristic odor; with ferric chloride it yields no red color; with silver nitrate its alkaline salts form a crystalline precipitate which is insoluble in cold water.

5. *Valerianic acid* boils at 176.3° C., and has a penetrating, disagreeable odor. Its silver salt crystallizes in plates, which are soluble with difficulty.

Cholesterin.—Cholesterin ($C_{26}H_{44}O$) occurs in small amounts in almost all animal fluids. It is found also in various tissues of the body, especially in the brain. Its origin and mode of formation in the various organs of the body, as well as the cause of its presence in the alimentary canal, are as yet unknown. It crystallizes in colorless, transparent plates, the margins and angles of which usually present a ragged appearance (Fig. 53). It is practically insoluble in water, dilute acids, and alkalies. In boiling alcohol it is readily soluble and crystallizes out from this solution on cooling; it is likewise easily soluble in ether, chloroform, and benzol.

In order to obtain cholesterin from the feces, in which it is always present, though rarely in crystalline form, the fatty acids, phenols, indol, and skatol must first be distilled off, as described, when the residue is strongly acidified with sulphuric acid, extracted with alcohol, and then with ether. The ethereal extract is filtered, the ether distilled off, and the residue digested with sodium carbonate, in order to transform into their salts any fatty acids which may still be present. This mixture is then evaporated to dryness, and again extracted with ether. The alcoholic extract above mentioned is also filtered, supersaturated with sodium carbonate, the alcohol distilled off, the residue dissolved in water and likewise extracted with ether. In the watery alkaline residue there remain bile-acids, oleic, palmitic, and stearic acids, which can be separated by transforming them into

Fig. 53.



Cholesterin crystals.

their barium salts. The cholesterin and fats pass over into the ether. This is distilled off and the residue treated with an alcoholic solution of potassium hydrate. The alcohol is evaporated on a water-bath, the remaining liquid diluted with water and again ex-

tracted with ether. The fats remain in the aqueous solution as soaps, while the cholesterin has passed into the other.

Tests for cholesterin: 1. Under the microscope add a drop of concentrated sulphuric acid to some of the crystals; they gradually disappear, the edges assuming a yellowish-red color.

2. Dissolve a few crystals in chloroform, add concentrated sulphuric acid, and shake the mixture: the chloroform assumes a blood-red to a purplish-red color, while the sulphuric acid at the same time shows marked fluorescence.

To isolate the fatty acids, the solution of soaps obtained above is acidified with dilute sulphuric acid, when the fatty acids which have separated out may be filtered off and identified individually by their boiling-points and the analysis of their barium salts.

The final filtrate, when neutralized with ammonium hydrate, contains glycerin.

The Biliary Acids.—The biliary acids found in the feces are: glycocholic acid ($C_{26}H_{43}NO_6$), taurocholic acid ($C_{26}H_{45}NSO_7$), and cholalic acid ($C_{24}H_{40}O_5$).

The two former occur normally in the bile, and can be decomposed into cholalic acid and glycocoll, and cholalic acid and taurin, respectively; as this process of decomposition takes place ordinarily in the intestines, the third acid—*i. e.*, cholalic acid—is always found in the feces.

In order to demonstrate the biliary acids, the fatty acids, phenols, indol, and skatol are first removed by distillation with phosphoric acid. The residue is taken up with water and boiled, and the filtered liquid precipitated with lead acetate and a little ammonium hydrate. The biliary salts of lead are contained in the precipitate, from which they can be removed by washing with water and finally boiling the precipitate with alcohol. The washings are filtered and the lead salts transformed into sodium salts by treating the filtrate with sodium carbonate. After further filtration the filtrate is evaporated to dryness and the residue extracted with hot alcohol. Upon evaporation the salts of the acids sometimes crystallize out as such, while more often a dirty amorphous precipitate is obtained, which may be rendered crystalline by treating with ether. The amorphous residue, however, can be employed for making the necessary tests.

Pettenkofer's Test.—A small amount of the substance is dissolved in water, and treated with two-thirds its volume of concentrated sulphuric acid, care being taken that the temperature does not exceed 60° or 70° C. While stirring, a 10 per cent. solution of cane-sugar is added drop by drop. If biliary acids are present, the solution assumes a beautiful red color, which on standing turns a bluish violet. This test depends upon the action of furfurol, derived from the sulphuric acid and cane-sugar, upon the biliary acids.

Pigments.—The principal pigment of normal feces is termed

stercobilin, and was first isolated from this source by Vanlair and Masius.[1] Owing to its great similarity to hydrobilirubin, it has even been regarded as identical with it, but Garrod and Hopkins[2] have now conclusively shown that whereas the urobilin of the urine and the stercobilin of the feces are identical in composition, as also in properties, they differ conspicuously from hydrobilirubin, and especially in the much smaller percentage of nitrogen which they contain, viz., 4.11, as compared with 9.22 per cent. It is derived from bilirubin, and formed in the upper regions of the large intestine more especially, as the result of bacterial activity.[3] This explains the observations that as a rule the meconium and the solid excreta of the first day or two of life contain no urobilin, and that the pigment also disappears, when for any reason the bile is prevented from entering the intestinal canal.

To isolate the pigment from the feces, the material is first extracted with alcohol. The alcoholic extract is evaporated to dryness; the residue is extracted with water, the aqueous solution acidified with sulphuric acid and saturated with ammonium sulphate, when on shaking with chloroform or a mixture of chloroform and ether the pigment is taken up by the organic solvent.

The free pigment is a brown amorphous substance of a characteristic odor, and melts at a temperature below 100° C. On cooling, it forms a brittle, shellac-like material, which is said to be quite characteristic. It is soluble in ether, chloroform, water, and amyl alcohol. On treating its solutions with zinc chloride and ammonia a beautiful green fluorescence is obtained. Such solutions then show three bands of absorption, of which the one between *C* and *F* is the most characteristic (see also Urinary urobilin).

Hæmatoporphyrin, to judge from recent investigations by Stokvis[4] and Garrod,[5] is likewise a normal component of the feces, but occurs only in traces. Garrod states that with Saillet's[6] method, the basis of which is extraction with acetic ether, after the addition of acetic acid, he invariably found traces, comparable with those which normally are present in the urine. He also states that he found considerably larger amounts of the pigment in the meconium, both in that expelled during the first day or two of life, and in that removed from the intestines of stillborn infants.

The presence of these normal traces has been referred by some to the ingested blood-coloring matter of red meat and vegetable chloro-

[1] Vanlair and Masius, Centralbl. f. d. med. Wiss., 1871, vol. ix. p. 369.

[2] F. G. Hopkins and A. E. Garrod, "On Urobilin," Jour. of Physiol., 1898, vol. xxii. p. 451.

[3] A. Schmidt, Verhandl. d. XIII. Congresses f. inn. Med., 1895, p. 320. Vaughan Harley, Brit. Med. Jour., 1896, vol. ii. p. 898. Macfadyen, Nencki, and Sieber, Arch. f. exper. Path. u. Pharmakol., 1891, vol. xxviii. p. 311.

[4] Stokvis, Nederl. Natuur-en Geneeskundig Congres, 1899, p. 378.

[5] Garrod, "The Urinary Pigments in their Pathological Aspects," Lancet, Nov. 10, 1900.

[6] Saillet, Rev. de Méd., 1896, vol. xvi. p. 542.

phyll. Garrod, however, finds that the hæmatoporphyrin does not disappear when these articles of diet are withdrawn, and while admitting that the ingested hæmoglobin and chlorophyll may possibly be converted, in part at least, into hæmatoporphyrin, he concludes that the greater portion is derived from human sources. On the whole, the evidence seems now in favor of the view that the hæmatoporphyrin which is found both in the urine and in the feces originates within the liver, and is eliminated into the intestinal canal in the bile (see also Hæmatoporphyrinuria).

Xanthin Bases.[1]—The amount of xanthin bases which is normally eliminated in the feces is about 0.11 gramme. They are largely represented by guanin, and adenin, while xanthin and hypoxanthin are the common forms which are met with in the urine.

PATHOLOGY OF THE FECES.

General Characteristics.

Number of Stools.—As has been pointed out (page 207), one or two stools in the twenty-four hours may be considered as normal. Individual peculiarities, however, must be taken into consideration.

As the consistence of the stools is altered in *diarrhœa*, this condition may be defined as one in which too frequent and liquid passages occur, while the reverse holds good for *constipation*, the consistence of the stools in this condition being usually also altered. The term *obstruction*, on the other hand, denotes a state of affairs in which no stools are voided. In a general way it may be said that whatever gives rise to increased peristalsis likewise produces diarrhœa, and that whatever diminishes peristalsis gives rise to constipation. In the former condition the number of stools may vary from one to thirty, forty, or even fifty in the twenty-four hours, as in Asiatic cholera. The consistence of the stool when only one is passed in the twenty-four hours will, of course, decide the question whether the case should be regarded as one of diarrhœa or not. One stool passed in the twenty-four hours may under certain conditions be regarded as a symptom of constipation, but more commonly this term is applied to a condition in which a stool occurs only every two, three, four, or more days.

Consistence and Form.—The consistence of the stools may undergo variations, which run a course parallel to their number. They may be thin, mushy, and even watery.

In constipation, on the other hand, owing to an increased absorption of water, the feces may be passed as very hard and perfectly dry, roundish, scybalous masses, the rotundity of which is undoubtedly referable to their long sojourn in the haustra of the colon. The

[1] M. Krüger u. A. Schittenhelm, Zeit. f. phys. Chem., 1902, vol. xxxv. p. 753.

individual scybala usually vary in size from that of a hazelnut to that of a walnut, and are frequently provided with one or two indentations which represent impressions of the tænia of the colon. Still smaller masses, closely resembling the dejecta of sheep, may also be seen. Their presence was formerly regarded as characteristic of stricture of the colon, but they are likewise found in ordinary cases of chronic constipation. Fecal ribbons and columns of the diameter of a pencil are found in cases of enterospasm of neurotic origin, as well as in stricture of the colon.

Amount.—The absolute amount of feces voided in the twenty-four hours bears an inverse relation to the number of stools and their consistence, providing, of course, that no abnormally large ingestion of food has occurred. In that case an abnormally large stool of moderate firmness may be passed. Two exceptions must, however, be noted to this rule—*i. e.*, the passage of large quantities of firm feces, following an attack of constipation of long duration or an attack of obstruction. Lynch[1] reports a remarkable instance in which, following a prolonged attack of constipation, an enema caused the evacuation of 20 kgrms. of fecal matter. Especially large amounts of feces are observed in cases of biliary obstruction, where 1100 grammes may be exceeded. In cases of fermentative dyspepsia the amount may also be large, varying between 400 and 900 grammes, while the patients are on a diet on which normal individuals would pass from 200 to 270 grammes in the twenty-four hours. Still larger amounts are noted in cases of enteritis. Schmidt mentions a case in which 2780 grammes were eliminated (these figures have reference to a three days' experiment with a test diet; see page 294).

Odor.—As the normal offensive odor of the feces is largely due to products of intestinal putrefaction, an increase in this respect will naturally be referable to conditions in which the putrefactive processes are increased. A most disagreeable odor is thus met with in the so-called acholic stools. The odor of fatty acids is observed in the lighter grades of infantile diarrhœa, while a markedly putrid odor is associated with its severer forms. A very characteristic, sperm-like odor is further noted in the stools of cholera, owing to the presence of considerable quantities of cadaverin. A truly rotten stench is present in the gangrenous form of dysentery, and in carcinomatous and syphilitic ulceration of the rectum. An ammoniacal odor is due to an admixture of urine undergoing ammoniacal decomposition.

Reaction.—The reaction of the stools is variable under pathological conditions, and of but little clinical interest. In typhoid fever an alkaline reaction is so constantly met with that this symptom might possibly be of value in doubtful cases. It may, however,

[1] R. Lynch, "Coprologia," Thesis, Buenos-Ayres, 1896, p. 38.

also be neutral, amphoteric, or even acid. In acute infantile diarrhœa an acid reaction is the rule, but exceptions also are not infrequent. Normal stools of sucklings are acid, the degree of acidity, according to Langstein,[1] corresponding to about 2.1 to 3.7 per cent. of normal NaOH for 100 grammes of the moist feces.

Color.—The color of the feces in disease may vary a great deal. When unaltered bile is present, the stools may assume a golden-yellow, a greenish-yellow, or even a green color. In cases of biliary obstruction or suppression, on the other hand, they become pasty and have a grayish or even a white color. This, however, is not so much due to the absence of coloring-matter derived from the bile, as to an insufficient absorption of fats, as was shown by Strümpell, who succeeded in obtaining stools of a light-brown color after feeding patients affected with catarrhal jaundice upon a diet containing minimal amounts of fat. *Such acholic or colorless stools*, as it would be better to say, are not only found associated with biliary obstruction, however, but may also occur when the ducts are patent. They have thus been observed in various cases of leukæmia, carcinoma of the stomach or intestine, in simple infantile enteritis, chronic nephritis, chlorosis, scarlatina, tubercular enteritis, and especially frequently in debilitated consumptives and in cases of chronic tubercular peritonitis in children. In some of these conditions, as in tuberculosis of the intestines and of the peritoneum, the lack of color is probably due to a diminished absorption of fats. In others, however, this explanation does not hold good, as abnormally large amounts of fat are not necessarily present. In such cases the lack of color is probably referable to the formation of colorless decomposition-products of bilirubin, such as the leuko-urobilin of Nencki, but nothing definite is known of the conditions which favor the formation of these products. In this connection it may be interesting to note that in those cases in which the biliary ducts are patent the color of the stools may vary not only from day to day, but even within the twenty-four hours.[1] A neurasthenic patient occurring in my practice thus passed an acholic stool almost every morning and usually colored feces in the afternoon, for a period of several weeks.

Generally speaking, the color of the stools becomes lighter the larger the number of movements, and *vice versa*. In Asiatic cholera and dysentery they may thus be colorless, while in severe constipation the scybalous masses are almost black.

If *blood* is present, the stools may present a scarlet-red, a dirty brownish-red, a coffee, or even a perfectly black color. *Adherent blood*, usually bright red in color and found on scybalous masses, is probably always derived from the rectum or anus, while a change in

[1] L. Langstein, Jahrbuch. f. Kinderheilk., vol. vi. Heft 3.

[2] Pel, Centralbl. f. klin. Med., 1887, vol. viii. p. 297. Le Nobel. Arch. f. klin. Med., 1888, vol. xliii. p. 285. Vogel-Biedert, Lehrbuch der Kinderkrankheiten, 9th ed., 1887, p. 115, Enke, Stuttgart. Berggrün u. Katz, Wien. klin. Woch., 1891, vol. iv. p. 858.

color, indicating an earlier date of the bleeding, usually points to the colon.

An *intimate admixture of blood* with the stool, the color being at the same time altered, so as to vary from a brownish red to black (owing to the presence of ferrous sulphide), is indicative of hemorrhage into the stomach or the small intestine. The darker the color of the blood the more remote from the anus will be, as a rule, the seat of the hemorrhage. Black or coffee-colored stools are thus observed in cases of ulcer of the stomach or of the duodenum, in melæna neonatorum, and similar conditions.

When profuse intestinal hemorrhages take place, however, as in some cases of typhoid fever and melæna, and particularly when diarrhœa exists at the same time, the blood which appears in the stools may be changed very little or not at all.

While, as a rule, simple inspection or a microscopical examination of the feces will determine whether or not blood is present, it may at times be necessary to resort to more delicate tests, as the hemorrhage may have been so slight as to escape detection with the naked eye, or so far removed from the anus that even blood-shadows cannot be found with the microscope. Hemorrhages of such trivial extent have been reported by Hässlin as occurring quite frequently in cases of chlorosis. This statement, however, I have not been able to confirm. If an investigation in this direction is to be made, the method of Müller and Weber (see page 261), or that of Korczynski and Jaworski, should be employed.

Korczynski and Jaworski's Test.—A small amount of the fecal material is treated with a pinch of potassium chlorate and a drop of concentrated hydrochloric acid. The mixture is carefully heated until it has become decolorized, more hydrochloric acid being added if necessary. The chlorine is then driven off, when one or two drops of a dilute solution of potassium ferrocyanide are added. In the presence of blood-coloring matter a distinct blue color is obtained, owing to the formation of Prussian blue.

For most purposes, however, the hæmin test will suffice, especially with the modification suggested by Strzysowski. The darkest particles of feces are boiled with a drop of a sodium iodide solution (1 : 500) and concentrated acetic acid. The resulting hæmin crystals are of a darker color than the common form (the hydrochlorate).

An admixture of *pus* in notable amounts also gives rise to a characteristic color, as is seen in cases of dysentery, syphilitic and carcinomatous ulceration of the colon and rectum, following the perforation of a parametritic or periproctitic abscess into the rectum, etc.

Carter and MacMunn[1] have recently pointed out that at times a chromogen may be present in the feces, which on exposure to the air is transformed into a red pigment, simulating blood-coloring matter. They report three cases in which this was observed. Mac-

[1] Carter and MacMunn, Brit. Med. Jour., 1899.

Munn expresses the opinion that the substance in question is closely related to stercobilin. The stools showed streaks of red upon the surface, and after further exposure and repeated agitation turned a pronounced blood red throughout.

Green stools are observed especially in infants, and may be referable to two different causes, being dependent on the one hand upon the presence of a bacillus, described by Le Sage, which produces a green coloring-matter, while on the other it may be referable to biliverdin. When green stools occur frequently, this condition is associated with the clinical symptoms of a severe cholera infantum. Such stools have also been noted in dysentery referable to an infection with the Bacillus pyocyaneus.

Quite characteristic also are the ipecacuanha stools, which closely resemble the so-called acholic stools. The green color produced by calomel, the yellow by santonin, rheum, and senna, the black by iron, manganese, and bismuth, have already been mentioned (see page 208).

Macroscopical Constituents.

Alimentary Constituents.—After having thus considered the number of stools, their consistence, reaction, odor, and color, it is necessary to look for gross admixtures, and especially for the presence of undigested food-material, such as pieces of meat, flakes of caseïn —this especially in the stools of children—and fragments of amylaceous food. The occurrence of such a condition, constituting what was formerly known as *lientery*, is always indicative of disturbed intestinal or gastric digestion, or both. It is, hence, observed in cases of chronic intestinal catarrh, febrile dyspepsia, following the use of cathartics, etc.

Occasionally also unaltered food in large amounts is found in the feces, owing to a direct communication between the stomach and the colon, as in cases of perforating ulcer or carcinoma of the stomach.

When fat is present in abnormally large amounts it can usually be recognized with the naked eye. To this condition the term *steatorrhœa* has been applied (see page 287). In typical cases the fat is seen in the form of whitish or grayish masses, varying in size from that of a pea to that of a walnut, which are more or less intimately mixed with the fecal material, and may at first sight be mistaken for flakes of caseïn. From these, it may be distinguished, however, by its chemical reactions and its peculiarly glistening appearance. In other cases stools may be seen in which the fecal column is covered, to a greater or less extent, with a grayish, dense, asbestos-like substance, while the core itself presents the usual color. Nothnagel states that this appearance is referable to congealment of the fat, when it is exposed to a lower temperature than that of the body. I have repeatedly observed this appearance, however, in stools which had just been voided and were still warm. The passage of liquid oil in the

absence of fecal material has also been recorded, but it seems doubtful that the oil in such cases entered the body by the mouth. Following the use of oil enemata such stools are, of course, seen.

The elimination of abnormally large quantities of fat may be due to the ingestion of correspondingly large amounts. More frequently, however, it is referable to distinctly pathological conditions. A steatorrhœa will thus naturally occur when an insufficient supply of bile is poured into the small intestine, and hence is observed constantly in cases of biliary obstruction. In these cases, however, the microscope is usually necessary to demonstrate the presence of the abnormally large quantities of fat. True steatorrhœa, on the other hand, viz., the presence of fat recognizable with the naked eye, is more commonly met with in diseases affecting the resorptive power of the small intestine, such as extensive atrophy or amyloid degeneration of the intestinal mucosa, tubercular ulceration, etc., or in diseases involving the integrity of the lymphatic glands and vessels of the mesentery, as in chronic tubercular peritonitis, caseous degeneration of the mesenteric glands, etc. In simple catarrhal conditions, however, steatorrhœa may also occur, and not only in infants, but, according to my experience, also in adults. The question whether or not steatorrhœa is constantly observed in cases of pancreatic disease, as some observers have claimed, may now be answered in the negative, although it must be admitted that the two conditions are very frequently associated. Le Nobel, who has recently investigated this subject, arrived at the conclusion that the steatorrhœa in itself is of little practical importance, but that its association with the absence of products of putrefaction from the stools, the absence of the salts of the fatty acids, and the presence of maltose in the urine, may possibly be regarded as indicating the existence of pancreatic disease.

Mucus and Mucous Cylinders.—So long as mucus occurs in small particles only, adherent to otherwise normal feces, it is of no pathological significance. Larger amounts are almost always indicative of a catarrhal condition of the colon or rectum, no matter whether the stool is otherwise normal or whether diarrhœa exists at the time. Peculiar formations are occasionally seen, viz., so-called *mucous cylinders*, which are passed in large or small fragments in a condition which has been described by Nothnagel as *enteritis membranosa* or *colica mucosa*.[1] Such masses, which at times measure a foot or more in length, are ribbon- or net-shaped, and are frequently passed in the absence of fecal matter, with severe tenesmus. They closely resemble Curschmann's spirals, but lack the central thread and the Charcot-Leyden crystals. They are probably indicative of

[1] Nothnagel, "Colica mucosa," Beiträge z. Physiol. u. Path. d. Darmes, 1884. Fleiner, Berlin. klin. Woch., 1893, Nos. 3 and 4. Einhorn, Arch. f. Verdauungskrank., vol. iv. p. 456.

chronic constipation associated with catarrh of the colon. Not to be confounded with this condition is the passage of masses of mucus, which do not present the cylindrical form, but which also may be passed with a great deal of tenesmus and in the absence of fecal matter; this is very commonly seen in cases of nephroptosis associated with gastroptosis and enteroptosis. These formations are in all probability also referable to a catarrhal condition of the colon. In cholera Asiatica particles of mucus are seen which resemble grains of rice; their presence was formerly regarded as characteristic of the disease; but they are now known to occur in ordinary catarrhal conditions also.

Biliary and Intestinal Concretions.—Most important from a diagnostic standpoint is the examination of the feces for the presence of biliary concretions, which should never be neglected in cases of colicky abdominal pain of doubtful origin, whether associated with jaundice or not.

When searching for gall-stones the feces should be stirred with water and passed through a fine sieve. Biliary concretions may then be found as small, crumbling masses or as hard stones presenting an irregular contour or the smooth, characteristic facets. In size they may vary from that of a millet-seed to that of a pigeon's egg; large stones are rarely passed by the bowel unless perforation has occurred into the intestines and usually into the colon.

Some calculi consist almost entirely of cholesterin, while others are composed essentially of inspissated bile, and still others of calcareous salts. The former are the most common, and are readily recognized by their softness and color, which may be white, grayish, bluish, or greenish. Their specific gravity is lower than that of water. Very frequently they contain a nucleus, composed of earthy sulphates or phosphates. An analysis which I made of a stone of this kind, weighing 10.548 grammes, gave the following results:

Cholesterin	72.590 per cent.
Mineral salts	0.247 "
Fats	5.090 "
Biliary pigments	13.930 "
Organic matter	7.270 "

Calculi which consist largely of biliary pigments are brown in color. They are hard, and heavier than water. Frequently they contain traces of copper and zinc (Fig. 54).

Calculi composed of calcareous salts generally present an irregular, roughened contour.

Within recent years Welch has drawn attention to the not infrequent presence of pure colonies of the Bacillus coli communis in gall-stones, apparently forming their nucleus. Typhoid bacilli also have since been observed in their interior, and it appears likely that the

formation of gall-stones is primarily referable to an invasion of the gall-bladder by such micro-organisms.

Analysis of Gall-stones.—The stone is finely powdered and dried at a temperature of 100° C. It is then extracted with boiling water and the washings concentrated upon a water-bath to about 100 c.c. One portion of this amount is evaporated to dryness, and the soluble residue, as well as the mineral ash, determined after desiccation at a temperature of 105° C. The other portion is likewise evaporated

FIG. 54.

Gall-stones.
a, cholesterin; *b*, pigment-stones.

to dryness and extracted with alcohol containing a small amount of ether, sodium glycocholate being thus obtained. After treatment with hot water, as described, the substance is successively extracted with alcohol and ether. In the alcoholic extract fats and a small amount of cholesterin will be found. The greater portion of this is in the ethereal extract. The residue, which is insoluble in hot water, alcohol, and ether, is treated with a moderately strong solution of hydrochloric acid, the earthy phosphates and oxides being thus obtained united to pigments. The bilirubin is removed by extracting with boiling chloroform. The pigments which are not dissolved in this manner are biliprasin, bilihumin, etc.

Intestinal concretions (enteroliths) are rare and usually come from the appendix. At times they contain some foreign body, such as a grape-seed, as a nucleus, upon which calcium and magnesium salts have become deposited.

Fecal calculi or *coproliths* are likewise only rarely seen. They represent inspissated fecal material which has become impregnated with lime and magnesium salts. More commonly they are found at the post-mortem table in the cæcum, in the haustra of the colon, and in the rectum.

Intestinal sand is also rare. In the German literature I have found reports of only three cases, while in the French literature about sixteen have been recorded. Of its origin nothing is known. The condition is commonly associated with enteritis membranacea. The material presents a brownish color, but may be light green. In one case reported by Deetz[1] it was possible to demonstrate the presence of calcium phosphate with traces of calcium oxalate.

[1] E. Deetz, Deutsch. Arch. f. klin. Med., 1901, vol. lxx, p. 365. See also Dieulafoy, "La lithiase intestinale et la gravelle de l'intestin," Presse méd., March 10, 1897 (extract in Centralbl. f. klin. Med., 1897, p. 904).

Microscopical Examination.

Technique.—In hospital work the stool should be passed into a well-warmed bed-pan and examined at once. This is particularly important in the search for amœbæ. In private practice patients should be instructed to send their stools to the physician as soon as possible, when suspicious-looking particles should be placed upon the warm stage or examined upon a well-warmed slide. A very convenient form of warm stage, which may be obtained from instrument-makers at low cost, is composed of brass and made to be held in position on the stage of the microscope by spring clips. It is about 8 cm. long and 3 cm. broad, and has cemented to a recessed bottom an ordinary glass slip; an opening measuring 1.35 cm. in diameter is in the centre of the stage. To one of the long sides of the brass stage is fitted a projecting stem, about 10 cm. long, to which the heat of a spirit-lamp is applied.

For ordinary purposes it is well to place the stool, if watery, in a conical glass, and to cover it with a layer of ether, so as to diminish the disagreeable odor. If mushy or firm, it should be spread upon a plate and covered with a layer of turpentine, or a 5 per cent. solution of carbolic acid or thymol.

Remnants of Food.—It has been pointed out that various microscopical remnants of food are observed in normal feces. In pathological conditions it is necessary to determine whether or not such remnants are present in abnormal amount, presupposing, of course, that excessive quantities of food have not been ingested. It is often possible to draw definite conclusions as to the state of intestinal digestion from the excess of one form of non-digested material over another. The presence of large quantities of undigested starch indicates a catarrhal condition of the small intestine, and it may, indeed, be said that the occurrence of more than traces of this material should always be regarded with suspicion. An increase in the number of muscle-fibres will, as a rule, likewise be observed under such conditions.[1]

Schmidt and Strassburger[2] have described a special form of intestinal fermentative dyspepsia, in which there is an isolated amylolytic insufficiency, which may be of functional or of organic origin (see Schmidt's fermentation test below).

In this connection it is noteworthy that in man extensive disease of the pancreas may exist without seriously disturbing amylolytic digestion.

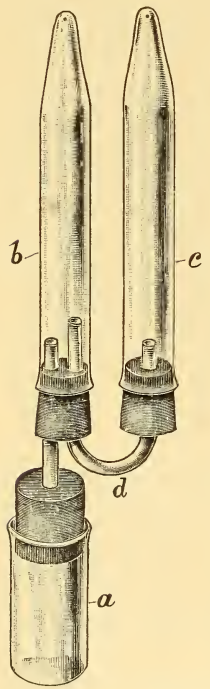
Schmidt's Fermentation Test.—To obtain a more exact insight into the degree of amylolytic insufficiency of the intestinal tract than

[1] Schmidt u. Strassburger, Deutsch. Arch. f. klin. Med., vol. lxix. p. 570.

[2] A. Schmidt, "Die Klinische Bedeutung der Ausscheidung von Fleischresten mit dem Stuhlgang," Deutsch. med. Woch., 1899, p. 811.

is possible from a microscopical study of the feces, Schmidt has proposed a special method which is based upon the continued digestion of the carbohydrates in the feces. The examination is made after the patient has been placed on the following test diet (Schmidt and Strassburger's test diet No. II.): milk, 1.5 liters; $3\frac{1}{2}$ eggs; strained oatmeal gruel (from 80 grammes of oatmeal); 100 grammes of Zwieback; 20 grammes of butter; 20 grammes of sugar; 125 grammes of steak (raw weight); and 190 grammes of potato (raw weight). The distribution of these various articles of food can be arranged as one chooses, or as follows: at 7.30 A.M., $\frac{3}{8}$ litre of milk and 2 Zwiebacks (each 33 grammes); at 10.30 A.M., $\frac{3}{8}$ litre of bouillon with $\frac{1}{2}$ egg; at 12 M. $\frac{3}{8}$ litre of milk with 1 egg; between 1 and 2 P.M. $\frac{1}{2}$ litre of oatmeal gruel (prepared from 40 grammes of oatmeal, 166 grammes of milk, 10 grammes of sugar, and $\frac{1}{2}$ egg); 100 grammes of well-done Hamburg steak (125 grammes of raw beef (raw weight) and 12 grammes of butter; 250 grammes of mashed potato (from 190 grammes of potato, 60 grammes of milk, and 8 grammes of butter); at 4.30 P.M. $\frac{3}{8}$ litre of milk, 1 egg, 1 Zwieback; at 7.30 P.M. $\frac{1}{2}$ litre of oatmeal gruel as at dinner-time. Before commencing with the test diet, however, it is necessary to demarcate the fecal material by giving a wafer or capsule containing 0.3 gramme of powdered carmine. The examination proper is made as soon as the feces are no longer colored red, viz., after from two to three days of the test diet. The necessary apparatus is pictured in the accompanying figure (Fig. 55), which represents one-third of the actual size. For each experiment 5 grammes of fresh fecal material are used (the feces being of medium consistence; otherwise a little more or less is taken, corresponding to about 1 gramme of dry residue). The material is well stirred with water in the bottle *a*, which is filled entirely and closed with the rubber stopper, care being taken to exclude bubbles of air. Tube *b* is filled with water from the tap and also closed without admission of air. Tube *c* should contain no water; it has a pin-hole aperture at the top. The communicating tube *d* is adjusted as shown in the figure. The apparatus is then placed in the incubator at 37° C. for twenty-four hours, not longer. During this time the carbohydrate fermentation will have been completed (Schmidt's Frühgährung). During the evolution of gas water will be dis-

Fig. 55.



Schmidt's fermentation tubes.

placed from *b* into *c;* the resulting column is measured and represents the degree of fermentation. The result is regarded as positive if more than a quarter tubeful of gas is obtained. With the test diet in question this would mean a condition approximating the normal. In such an event the patient is placed for two days further on test diet No. I., which differs from No. II. only in the absence of the meat and potato. If then there is still a positive result, the diagnosis of "fermentative dyspepsia" is justifiable. In order to eliminate errors arising from possible formation of gas as the result of albuminous putrefaction the fermenting fecal material should be tested from time to time in a control specimen. If the formation of gas is due to carbohydrate fermentation, there will be an increasing degree of acidity (tested with litmus-paper); this increase, however, is not always marked; at any rate, there must be no increasing alkalinity.

The so-called *acholic stools* are usually very rich in fat, and particularly so in cases of biliary obstruction associated with jaundice. At other times the lack of color, as has been mentioned above, is not referable to the secretion of an insufficient amount of bile, but to the presence of colorless decomposition-products of bilirubin, such as the leuko-urobilin of Nencki. In these cases abnormally large quantities of fat are not always present. The conclusion that a stool contains excessive amounts of fat because it is apparently acholic is hence not justifiable unless a microscopical examination has been made.

Leiner's Test for Casein.—Caseïn is most conveniently demonstrated with Leiner's method. To this end, a small amount of fecal matter is spread on a slide and dried in the air. It is then fixed by heat—passing the specimen through the flame of a Bunsen burner three or four times is sufficient—and stained with a mixture of equal parts of a 0.75 per cent. solution of acid fuchsin and methyl-green in 50 per cent. alcohol, the mixture being diluted ten times with water. After fifteen minutes the preparations are placed in distilled water and allowed to remain for one hour or longer. Caseïn and paracaseïn are thus stained a pale blue or violet, while similar bodies are practically all colored a light green, or more rarely a yellowish green.

Epithelium.—Epithelial cells when present in large numbers always indicate an inflammatory condition of some portion of the intestinal tract.

Cylindrical epithelial cells are found in abundance in all inflammatory conditions affecting the intestinal mucosa. They are almost exclusively seen imbedded in mucus, and it is interesting to note that the cloudy appearance of the mucus is referable to the presence of these elements, and not to leucocytes, as is the case in the sputum. When bile-stained specimens are met with, the conclusion is justifi-

able that the small intestine is involved. Degenerative forms are mostly seen ; well-preserved cylindrical or goblet-cells may, however, also be found, and are, according to my experience, much more common than is generally supposed.[1]

Epithelioid cells may be found in carcinoma of the rectum.

Red Blood-corpuscles.—Unaltered red blood-corpuscles, according to Nothnagel, are but rarely observed in the feces, no matter how intensely red they may be colored, providing that an ulcerative process affecting the colon or the rectum can be excluded ; in that case, as in the severer forms of dysentery, large numbers may be observed. If the hemorrhage has occurred higher up in the intestine, large and small masses of a brownish-red color are seen, which consist of hæmatoidin. They are mostly amorphous, but in some specimens the characteristic rhombic crystals may be observed. In general, it may be said that the higher the seat of the hemorrhage the darker will be the color of the pigment, and the less the chances of finding well-defined red corpuscles. In such cases recourse must be had to the hæmin test (page 39), to the iron test of Korczynski and Jaworski (page 288), or to Donogany's test (page 262).

Mucus.—Small hyaline particles of mucus, visible only with the microscope, are not infrequently met with under pathological conditions, and are of distinct diagnostic significance. When bile-stained, their presence is always indicative of disease of the small intestine proper, while colorless particles point to a catarrhal condition of the upper portion of the large intestine or the lower portion of the small intestine. Beginners should be careful not to mistake apparently hyaline particles of vegetable residue for mucus. Mucus never yields a blue color when treated with iodine, or iodine and sulphuric acid, and examination with a higher power will show the entire absence of any definite structure. Both forms, viz., colorless and colored particles, are found intimately mixed with the feces, and may be very abundant. In addition to these forms Nothnagel has described the occasional occurrence of large numbers of roundish or irregular, very pale hyaline or opaque formations, which are devoid of all structure. Some specimens are homogeneous, while others present a distinct rimous appearance. They have thus far been found only in liquid stools, and are apparently of no diagnostic significance. To judge from their optic behavior, they probably consist of mucus.[2]

Leucocytes.—The presence of a large number of leucocytes usually indicates a severe catarrhal, if not an ulcerative, condition of the intestines, the number of leucocytes or pus-corpuscles standing in a direct relation to the intensity of the inflammatory process. Pure pus in large amounts is observed especially in dysentery, and in

[1] Nothnagel, loc. cit., page 226.
[2] A. Schmidt, "Ueber Schleim im Stuhlgang," Zeit. f. klin. Med., vol. xxxii. p. 260.

cases in which accumulations of pus have perforated into the gut from adjacent organs or cavities.

Crystals.—The crystals which may occur in the feces have already been briefly considered (page 274). Of these, the so-called Charcot-Leyden crystals deserve more detailed consideration. While occurring at times in normal stools, as also in those of typhoid fever, dysentery, and phthisis, such observations are rare. They appear to be quite constantly present, on the other hand, in cases of anchylostomiasis and anguilluliasis. They are also frequently associated with Ascaris lumbricoides, Oxyuris vermicularis, Tænia solium and saginata. In cases of Trichocephalus they are but rarely seen, while they are always absent in the case of Tænia nana. These observations, made by Leichtenstern, are very important, and, according to the same observer, the occurrence of Charcot-Leyden crystals should always excite suspicion as to the existence of helminthiasis and lead to a careful examination of the feces for parasites or their ova. Their persistence in the feces after the evacuation of what would appear to be a complete tænia should be regarded as indicating the non-removal of the head. In amœbic colitis these crystals have also been observed by Lewis, Lafleur, Amberg, myself, and others.[1]

Animal Parasites.

I.—Protozoa:
- 1. Rhizopoda,
  - Monera,
  - Amœbina: Amœba coli.
- 2. Sporozoa, S. gregarina,
  - Coccidia,
  - Plasmodium malariæ.
- 3. Infusoria,
  - *a.* Ciliata,
    - Holotricha: Balantidium coli.
  - *b.* Flagellata.
    - Monadina,
      - Cercomonadina: Cercomonas.
    - Isomastigoda.
      - Tetramitina: Trichomonas.
    - Polymastigina: Megastoma.

II.—Vermes:
- Platodes,
  - Cestodes,
    - Tænia saginata.
    - Tænia solium.
    - Tænia nana.
    - Tænia diminuta.
    - Tænia cucumerina.
    - Bothriocephalus latus.
    - Krabbea grandis.

[1] Leichtenstern, Deutsch. med. Woch., 1885, vol. xi. Nos. 29 and 30; Ibid., 1886, vol. xii. Nos. 11–14; Ibid., 1887, pp. 565, 594, 620, 645, 669, 691, and 712.

II.—Vermes (*continued*):
- Trematodes,
  - Distoma hepaticum.
  - Distoma lanceolatum.
  - Distoma Buskii.
  - Distoma sibiricum.
  - Distoma spatulatum.
  - Distoma conjunctum.
  - Distoma heterophyes.
  - Amphistoma hominis.
  - Distoma hæmatobium.
  - Distoma pulmonale.
- Annelides,
  - Nematodes,
    - Ascarides,
      - Ascaris lumbricoides.
      - Ascaris mystax.
      - Ascaris maritima.
      - Oxyuris vermicularis.
    - Strongyloides,
      - Anchylostomum duodenale.
    - Trichotrachelides,
      - Trichocephalus hominis.
      - Trichina spiralis.
    - Rhabdonema strongyloides,
      - Anguillula intestinalis.

III.—Insecta:
- Piophila casei.
- Drosophila melanogastra.
- Homialomyia.
- Hyodrothœa meteorica.
- Cystoneura stabulans.
- Calliphora erythrocephala.
- Palleuria rudis.
- Lucilia cæsar.
- Lucilia regina.
- Sarcophaga hæmatoides.
- Eristalis arbustorum.
- Anthomyia.

Protozoa.—The *rhizopoda* are essentially characterized by the fact that locomotion does not take place by the aid of independent organs, but by means of pseudopodia, viz., protoplasmic processes which the animal is capable of protruding from any portion of its body. Six orders have been described by zoölogists, but only one, or possibly two, have thus far been found in the feces.

Whether or not representatives of the *monera* occur in the feces of man is still an open question. If so, they are apparently of no pathological significance.[1]

Of the *amœbina*, on the other hand, a most important member has been found, viz., the Amœba coli (Lösch).

The history of the discovery of this parasite and its relation to those severe forms of tropical dysentery and liver-abscess which are met with also in our more temperate zones is of much interest, and at the same time illustrates the great importance which attaches to a systematic examination of the feces in all aggravated forms of diarrhœa.

[1] Nothnagel, loc. cit., p. 110. Grassi, cited by Bizzozero. v. Jaksch, Wien. klin. Woch., 1888, vol. i. p. 511.

Amœba coli (Lösch).—In 1875 Lösch[1] discovered in the stools of dysenteric patients actively moving cell-like bodies of a roundish, pear-shaped, oval, or irregular form. He did not regard these as the cause of the disease, however, but looked upon them as only accidentally present. Similar bodies were observed in Hong-Kong by Normand in cases of colitis; and also by v. Jaksch. Sansino found them in a case in Cairo, and Koch in East Indian dysentery. It is interesting to note that Koch was the first to suspect the existence of a definite relation between dysentery and these organisms. Cunningham claims to have found amœbæ frequently in the stools of cholera patients at Calcutta, and Grassi in normal stools, but espe-

FIG. 56.

Amœba coli.

cially abundant in cases of chronic diarrhœa. Whether all these observations are correct, and whether the organisms observed were identical in all cases, is, of course, difficult to say. So much is certain, that the subject was still a very unsettled one when Kartulis[2] announced "that dysentery, and tropical liver-abscess associated with dysentery, are caused by the presence of the Amœba coli," basing his conclusion upon an examination of five hundred cases. The fact that this parasite was absent in all other intestinal diseases, such as typhoid fever, intestinal tuberculosis, the ordinary forms of diarrhœa, etc., speaks strongly in favor of Kartulis' view.

In perfect accord with these observations are those made at the Johns Hopkins Hospital.[3] Osler[4] was the first in this country to

[1] Loesch, "Massenhafte Entwickelung v. Amöben im Dickdarm," Virchow's Archiv, vol. lvi.

[2] Kartulis, "Zur Aetiologie d. Dysenterie in Egypten," etc., Virchow's Archiv, 1885, vol. cv., and 1889, vol. cxviii. Centralbl. f. Bakt. u. Parasit., 1890, vol. vii.

[3] Councilman and Lafleur, "Amœbic Dysentery," Johns Hopkins Hosp. Rep., 1891, vol. ii. C. E. Simon, Johns Hopkins Hosp. Bull., 1890.

[4] Osler, Johns Hopkins Hosp. Bull., 1890.

demonstrate the presence of the Amœba coli in a case of liver-abscess, both in the pus of the abscess and in the stools. Stengel, Musser, Dock, and others confirmed these observations, so that the pathogenic character of the Amœba coli may now be regarded as an established fact.[1] This statement is based not only upon the few facts, more historical in character than otherwise, which have just been detailed, but rather upon the *ensemble* of collected data, among which the absence of micro-organisms other than the amœba in the pus of the liver-abscesses, and the constant presence of the latter in such cases, rank among the most important. It is to be noted, however, that different forms of tropical dysentery exist, and that the Amœba coli is essentially associated with the more chronic form, while the acute types are probably of bacillary origin (see Shiga's bacillus).

The size of the amœbæ varies from 10 μ to 20 μ. When at rest their outline is, as a rule, circular, occasionally ovoid; but when in motion they present the extremely irregular contour of moving amœboid bodies (Fig. 56). The protoplasm can be differentiated into a translucent, homogeneous ectosarc or mobile portion, and a granular endosarc, containing the nucleus, vacuoles, and granules. Within the endosarc the vacuoles constitute the most striking feature. Sometimes the interior seems to be made up of a series of closely set clear vesicles of pretty uniform size. As a rule, one or two larger vacuoles are present, the edges of which are not infrequently surrounded by fine dark granules. True contractile vesicles displaying rhythmic pulsations have not been observed, although the vacuoles may at times be seen to undergo changes in size. In some the nucleus is quite distinct, while in others it may be altogether invisible. The protoplasm of the amœbæ is strongly basophilic. The organism can be cultivated artificially on hay-infusion in the presence of bacteria (Miller).[2]

Most distinctive are the movements of these bodies. From any part of the surface a rounded, hemispherical knob will project, and with a rapid movement the process extends and the granules in the interior flow toward it. In these movements the clear ectosarc seems to play the most important part.

The *Flagellata s. mastigophora* differ from the rhizopoda in being provided with from one to eight flagella, which serve as organs of locomotion and possibly also for the apprehension of food-particles. Representatives of two orders only, viz., the *monadina* and *isomastigoda*, have been found in the feces. Of the monadina in turn only one family, viz., the cenomonadina, and of the isomastigoda only two families, the tetramitina and polymastigina, are represented.[3]

[1] For the more recent literature see especially H. F. Harris, "Amœbic Dysentery," Am. Jour. Med. Sci., 1898, p. 384.

[2] C. O. Miller, "The Cultivation of Amœbæ," Contributions to the Science of Medicine, by the pupils of W. H. Welch, Baltimore, 1900, p. 511.

[3] W. Janowski, Zeit. f. klin. Med., vol. xxxi. p. 445.

The *cenomonadina* are small, oval, frequently elongated bodies, provided with one long flagellum at the anterior end, at the base of which food-vacuoles are situated. At the posterior end amœboid movements may be observed, and there can be no doubt that the taking up of food, to some extent at least, also occurs by the aid of pseudopodia. To this family belongs the *cercomonas* of Davaine and Lambl. The tetramitina are small, elongated bodies, provided with four flagella and a lateral, undulating membrane, which was formerly mistaken for a posteriorly directed flagellum. The tail-end of the organism tapers to a point. The nucleus is located at the base of the flagella. To this family belongs the parasite which was first discovered by Donné in the vagina, and which later was found also in the feces, and which has been variously designated as Trichomonas hominis, Cercomonas coli hominis, etc.

The *polymastigina* are small, somewhat oval bodies, provided with two or three flagella, situated either anteriorly or laterally—two or three on each side—while at the same time two additional flagella issue from the posterior end, which may either be rounded off or taper to a point. To this family belongs the Megastoma entericum of Grassi.

Only three parasites belonging to the order of the flagellata have thus far been encountered in the human feces, viz., the Cercomonas hominis of Davaine and Lambl, the trichomonas of Donné, and the Megastoma entericum of Grassi. To judge from the earlier literature upon the subject, many others have also been found, but more modern investigations have shown that they are in reality identical with the three just mentioned. The question whether or not these flagellate bodies are of pathological importance still remains *sub judice.* They are apparently met with only in diseases associated with diarrhœa, and it appears that in some cases at least this is directly dependent upon their presence; in others the impression is gained as though they merely maintained an already existing diarrhœa referable to other causes; while in a third class of cases no relation can be discovered between their presence and the disease in question. Cohnheim[1] has recently pointed out that living infusoria in the feces may be a symptom of a primary chronic stomach affection (gastritis, usually the atrophic form). According to the same writer, encysted infusoria may also be found in the feces of healthy individuals, but in such cases we may assume that at some time previously a gastritis or a gastro-enteritis has existed. He thinks they have no pathogenic significance, and are merely of symptomatic-diagnostic interest.

Cercomonas of Davaine-Lambl: *syn.*, Cercomonas hominis (Davaine); monas (Marchand); Monas lens (Grassi); Monas monomitina (Grassi). The adult organism (see Fig. 57) is oval or

[1] P. Cohnheim, Deutsch. med. Woch., 1903, vol. xxix. p. 248.

roundish in form, and provided anteriorly with a single long flagellum and posteriorly with a tail-like appendage. Its length varies from 0.005 to 0.014 mm. The younger forms are pear- or S-shaped, and sometimes irregular in outline; the flagellum is either absent or only rudimentary.

Fig. 57.

Cercomonas intestinalis.

a, Cercomonas of Davaine, after Leuckart; *b*, Cercomonas intestinalis, after Lambl; *c*, *d*, same, ordinary forms; *e*, *f*, same, well-developed forms; *g*, *h*, *i*, same, degeneration-forms; *k*, *l*, same, abortive forms.

Upon prolonged observation it will be seen that the adult parasite loses its flagellum and may protrude a protoplasmic process instead, while vacuolation occurs at the same time, indicating approaching death.[1]

Trichomonas, Donné: *syn.*, Trichomonas vaginalis (Donné); Trichomonas hominis (Grassi); monocercomonas (Grassi); cimænomonas (Grassi); Protorycomyces coprinarius (Cunningham and Lewis); Cercomonas coli hominis (May); Trichomonas intestinalis (Leuckart and Roos); Cercomonas s. Bodo urinarius (Künstler). The parasite (Fig. 58) is oval or spindle-shaped and measures from 0.012 to 0.03 mm. in length by 0.01 to 0.015 mm. in breadth. From its anterior pole four flagella are given off, which are almost as long as the organism itself. From this point an undulating membrane extends laterally to the posterior pole, which may be rounded off or tapers to a tail-like appendage. This membrane is best seen when the movements of the flagella have ceased, as in specimens fixed in mercuric chloride solution (1 : 5000). The nucleus is situated at the base

[1] Lambl, Prag. Vierteljahr., 1859, vol. lxi. p. 1. Davaine, Traité des entozoaires, 1860, Paris. Marchand, Virchow's Archiv, 1875, vol. lxiv. p. 293. Zunker, Deutsch. Arch. f. prakt. Med., 1878.

of the flagella, but is usually visible only in stained specimens (methylene-blue). At times the organisms may be observed to

FIG. 58.

Trichomonas intestinalis.

a, *a'*, *c*, trichomonas of the urine, after Marchand; *b*, Trichomonas vaginalis, after Donné; *b'*, same, after Scanzoni and Kölliker; *d*, Trichomonas intestinalis, after Piccardi; *e*, *e'*, *e''*, same, amœboid forms; *f*, *f'*, trichomonas of the urine, after Dock.

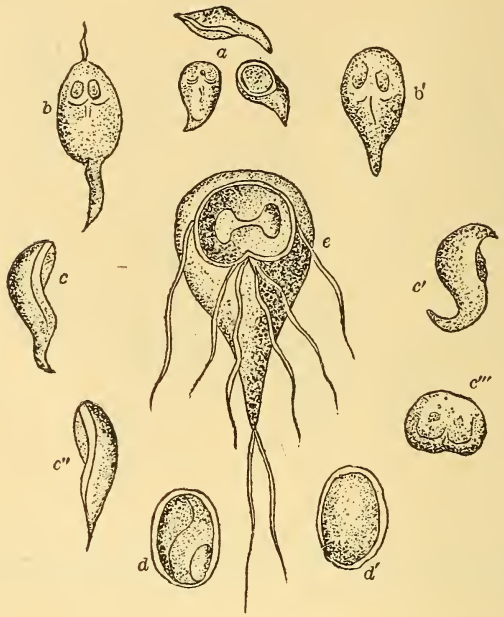
assume an amœboid form; the movements of the flagella have then ceased, and pseudopodia-like processes are protruded. The parasite is identical with the trichomonas which has been found in the vagina and in the urine.[1] When present in the feces the organism is usually seen in large numbers. Not infrequently it is found associated with other intestinal parasites.

Megastoma entericum, Grassi: *syn.*, Cercomonas intestinalis (Lambl); Megastoma intestinale (Bütschli); Lamblia intestinalis (Blanchard); Dimorphus muris (Grassi). The parasite (Fig. 59) is pear-shaped, and measures from 0.01 to 0.021 mm. in length by 0.0075 to 0.05 mm. in breadth. In its anterior portion a more or less well-marked depression can be made out, which constitutes the peristome or mouth-opening of the organism. It is provided with eight flagella, grouped in pairs. The first pair originates on the sides of the peristome and is directed backward. The second and third pair are situated somewhat posteriorly and are likewise directed backward, while the fourth pair issues from the tapering tail-end of the body.

[1] Marchand, loc. cit. Zunker, loc. cit., p. 236. Mosler u. Peiper, Nothnagel's Spec. Path. u. Therap., 1894, vol. vi.

In fresh specimens the eighth flagella can usually not be made out, as the third and fourth pair are frequently agglutinated. The best results are obtained when the organism has been killed with mercuric chloride solution. The individual flagella vary from 0.009 to 0.014

FIG. 59.



Megastoma entericum.

a, b, b', c, c', c'', c''', various forms of Cercomonas intestinalis, after Lambl; *d, d'*, encysted forms of Megastoma entericum, after Grassi and Schewiakoff; *e*, Megastoma entericum, adult form.

mm. in length. In the anterior portion of the peristome two round, hyaline bodies can be recognized, which represent nuclei. Vacuoles are absent, and nutrition occurs through osmosis, the parasite adhering to epithelial cells by its peristome. When treated with fixing solutions the chitinous envelope can be readily recognized. In the encysted form the organism is oval and measures from 0.007 to 0.1 mm. in diameter.

Grassi observed the organism in mice, rats, cats, dogs, rabbits, and sheep.[1]

Balantidium coli, Stein : *syn.*, Paramœcium coli (Malmsten). The organism is oval and measures from 70 μ to 110 μ in length by 60 μ to 72 μ in breadth. It is covered entirely with fine, actively motile cilia, which are grouped most densely about the funnel-shaped mouth, while at the anus only a few are seen. An ectosarc and an endosarc may be distinguished, and the parasite possesses the power

[1] Grassi u. Schewiakoff, Zeit. f. wiss. Zoologie, 1888, vol. xlvi. p. 143.

to change its shape, and may appear quite round. In its interior we find a large, somewhat kidney-shaped nucleus, two contractile vesicles, and frequently fat-droplets, starch-granules, etc.

The parasite is probably pathogenic, but comparatively uncommon outside of Sweden, Finland, and Russia. 90 cases have thus far been reported (1903). Of these, 25 occurred in Sweden, 14 in Finland, 24 in Russia, 11 in Germany, 5 in Italy, 1 in the Sunda Islands, 2 in the United States, 6 in Cochin China, 1 in Africa, and 1 in the Philippines. Infection occurs through the dejecta of swine.

Strong and Musgrave report that in their case blood examination showed a relative increase of the eosinophiles.

From 200 to 300 organisms have been encountered in a single drop of the liquid feces.[1]

The fourth class of protozoa, viz., the *Gregarina* or *sporozoa*,[2] is also said to be represented in the human feces. The coccidia and psorosperms belong to this order. They are oval bodies, measuring about 0.022 mm. in length, and contain in their interior a large number of small nuclei arranged in groups. They are entirely devoid of organs of locomotion, and obtain their nutriment by endosmosis. Reproduction occurs in a common capsule, which bursts at a certain time and sends forth a whole generation of fully developed organisms. In human pathology they have become of interest in so far as certain observers have ascribed to them a *rôle* in the etiology of neoplasms. A disease of the liver analogous to the *psorospermiasis* of rabbits has also been described in man, and parasites belonging to the same order have been observed in the skin.

In this connection I wish to refer to the occurrence of Laveran's *Plasmodium malariæ* enclosed in red corpuscles, in the stools of cases of malarial colitis. In one case of chronic malarial intoxication with dysenteric symptoms the diagnosis was first made after an examination of the stools for amœbæ; these were absent, however, while a number of plasmodia could be demonstrated, pointing to the probable nature of the colitis.

Vermes.—The class vermes has interested physicians since time immemorial, and is referred to in the writings of Hippocrates and others, special mention being made of the ascarides, called lumbrices, and the platodes, called lati. Speaking of the former, Lucas Tozzi, in 1686, says: "They find their way into the heart and its pericardium, into the brain, the lungs, the veins, and gall-bladder, where they are difficult to 'catch.'" The same author, speaking of their effects upon the body, enumerates the following conditions as caused by their presence: epilepsy, vertigo, sopors, delirium, convulsions,

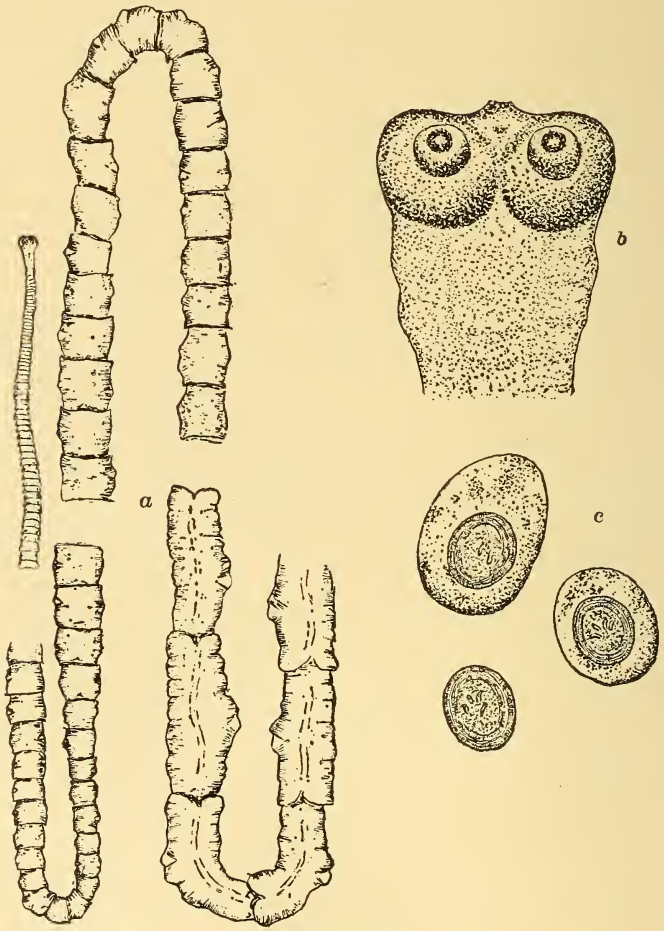
[1] Malmsten, Virchow's Archiv, 1897, vol. xii. p. 302. Sievers, "Ueber Balantidium Coli im menschlichen Darmkanal," Arch. f. Verdauungskrank., vol. v. p. 445. Janowski, "Balantidium Coli," Zeit. f. klin. Med., vol. xxxii. p. 303. Henschen, Arch. f. Verdauungsk., 1901, vol. vii. p. 501. Solorojew, Centralbl. f. Bacter., 1901, vol. xxix. pp. 821 and 849. A. Ehrenrooth, Zeit f. klin. Med., 1903, vol. xlix. p. 321.

[2] v. Wasielewski, Sporozoenkunde, 1896.

headache, syncope, palpitations, feelings of anxiety, cough, vomiting, nausea, diarrhœa, hiccough, prickling, borborygmi, erosions, tabes, acute and chronic fevers, and innumerable other maladies.

It was even then deemed very important to make a diagnosis before the administration of an anthelmintic—a point which is well

FIG. 60.



Tænia saginata.
a, natural size; *b*, head much enlarged; *c*, ova much enlarged.

to bear in mind at the present day, and the eggs, segments, or parasites themselves should be sought for in every suspected case before treatment is begun.

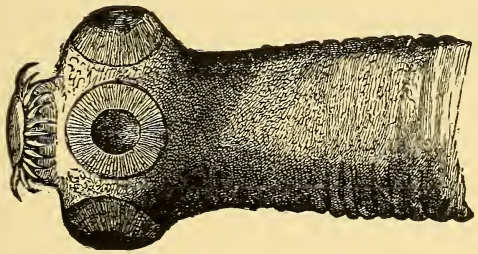
Tænia saginata, Goeze: *syn.*, T. mediocanellata (Küchenmeister); T. incruris (Huber); T. dentata (Nicola). This parasite (Fig. 60)

is the most common tapeworm both in Europe and North America. Infection occurs through the ingestion of measly beef. Its length varies from 4 to 8 m. The head, which is devoid of a rostellum, is surrounded by four pigmented suckers, each of which is encircled by a dark line. The individual segments are quite thick and opaque, and diminish in length as the head is approached, the largest measuring from 2 to 3 cm. They are each provided with a very much branched uterus, which opens laterally, the primary branches numbering about twenty on each side. The ova are elliptical in form, of a brown color, and usually enclosed in a distinct vitelline membrane. Upon careful observation a double contour with delicate radiating striæ can be discerned. In the interior the embryos are seen imbedded in a brown, granular material.

The larval form of Tænia saginata, the so-called Cysticercus tæniæ saginatæ (Leuckart), or the Cysticercus bovis (Cobbold), has been encountered in cattle, the Rocky Mountain "antelope," the llama, and the giraffe. In the human being it has not as yet been observed.[1]

Tænia solium, Rudolphi: *syn.*, T. cucurbitina, plana, pellucita, Goeze. This parasite (Fig. 61) is far less common in this country than

FIG. 61.



Head of T. solium. × 45. (LEUCKART.)

the Tænia saginata, and may indeed be regarded as a curiosity. In Germany, also, it is only rarely met with now, while formerly it was the most common tapeworm in that country. This change is undoubtedly owing to the fact that raw pork is now eaten less frequently. In Asia and Africa it is more common.

Tænia solium is usually much shorter than Tænia saginata, rarely exceeding 3.5 m. in length. Most characteristic is the head, which is provided with four pigmented suckers and a rostellum, furnished with from twenty-four to twenty-six hooklets arranged in a double row. The mature segments measure from 1 to 1.5 cm. in length

[1] J. Ch. Huber, Die Darmcestoden des Menschen. Bibliograph. d. klin. Helminthol., Heft 3, No. 4, p. 69, München, 1892. R. Leuckart, Die Parasiten des Menschen, etc., 2d ed., 1880, Pt. 1.

by 6 to 7 mm. in breadth, and contain a uterus which has only five to seven branches, thus differing greatly from that of Tænia saginata. The ova are round, of a brownish color, and surrounded with a thick, radially striated membrane; in their interior the hooklets of the embryos can usually be made out.

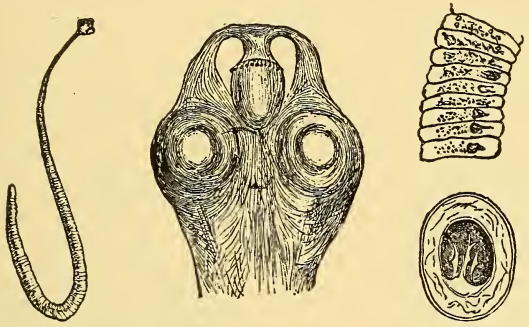
The larval form of this tapeworm, the *Cysticercus cellulosæ*, has been found in swine, the wild boar, in monkeys, in the brown bear, in the dog, etc. At times, though rarely, an auto-infection with the proglottides of Tænia solium has also been observed in the human being. Under such conditions the embryos of the worm are set free in the stomach, and may then migrate into various parts of the body, where they become encysted. Most commonly the cysticerci are found in the skin; they have, however, also been observed in the heart, the lymph-glands, liver, bones, tongue, spinal canal, the brain, and the eyes. I have had occasion to observe a case of this kind at the Johns Hopkins Hospital (reported by Osler). The patient, a laboring-man, had never worked as a butcher or a cook, and never had a tapeworm. The cysticercus nodules, which were situated between the skin and the fascia, were very numerous, seventy-five being counted on one day. One of these nodules was removed for examination, and was shown to be referable to the cysticercus of Tænia solium. The only subjective complaints in this case were pains and stiffness in the arms and legs. The individual cysticercus was elliptical or roundish in form, measuring from 1 to 10 mm. in diameter. In its interior the characteristic hooklets were seen.[1]

Tænia nana, v. Siebold: *syn.*, hymenolepsis (Weinland). This parasite (Fig. 62) seems to be the most common tapeworm of Italy and Egypt. It has also been seen in Buenos-Ayres, in Bangkok, Siam, and a few isolated cases have been reported in England and in Germany. In the United States the parasite seems to be not at all uncommon, but has probably been overlooked in many cases. Stiles states that in his laboratory eighteen cases have been diagnosed within a year (1902). It is found especially in young people, and often causes severe nervous symptoms, such as convulsions, loss of consciousness, and even melancholia. It is only 8 to 25 mm. long and 0.5 mm. broad. The head is ball-shaped and provided with four suckers and a rostellum, bearing twenty-four to twenty-eight hooklets arranged in a single row along its anterior edge. The individual segments are of a yellowish color and about four times as broad as long. The uterus is oblong and contains numerous ova, which are colorless, oval, and surrounded by a distinct non-striated membrane. They measure from 0.839 to

[1] Huber, loc. cit. Leuckart, loc. cit.; and Blanchard, Traité de Zoologie médicale, vol. i., Paris. The Inspection of Meats for Parasites, Bull. No. 19, Bureau of Animal Industry, Washington, 1898.

0.060 mm. in size. In their interior the embryonic worm, provided with five or six hooklets, may be distinguished. The number of worms which may at times be found in the digestive tract is most astonishing, 5000 and even more having been counted on several occasions. The cysticercus stage occurs in snails, which are frequently eaten raw in Egypt and Italy. Tænia nana has been identified with the Tænia murina of rats and other rodents.[1]

Fig. 62.



Tænia nana. Head, with rostellum drawn in ; proglottis; egg. (v. Jaksch.)

Tænia diminuta, Rudolphi : *syn.*, Tænia flavapunctata (Weinland); Tænia minima (Grassi); Tænia varerina (Parona); Tænia leptocephala (Creplin). Tænia diminuta was first described in man by Leidy, Grassi, and Parona. It measures 20 to 60 mm. in length, and is armed with two suckers, but is without a rostellum. The ova resemble those of Tænia solium. The cysticercus occurs in certain caterpillars and cocoons. In man it has been found in only six instances.[2]

Dipylidium caninum, Linné : *syn.*, Tænia canina (Linné); Tænia moniliformis (Pallas); Tænia cucumerina (Bloch); Tænia elliptica (Batsch). The parasite is found almost exclusively in children, infection occurring through dogs and cats. In the United States the disease is apparently rare. The only case reported is that of Stiles.[3] The larval form is found in lice and fleas. The worm itself measures from 15 to 35 cm. in length. The head is small, globular; the rostellum club-shaped with 3 or 4 transverse rows of hooks (about 60 in number) of rose-thorn form; anterior hooks 15 μ, posterior hooks 6 μ; suckers relatively large, rather elliptical. Segments 80–120 in number; gravid segments 8–11 mm. long, 1.5–3 mm. broad; often reddish-brown in color. Genital pores at equator or in posterior

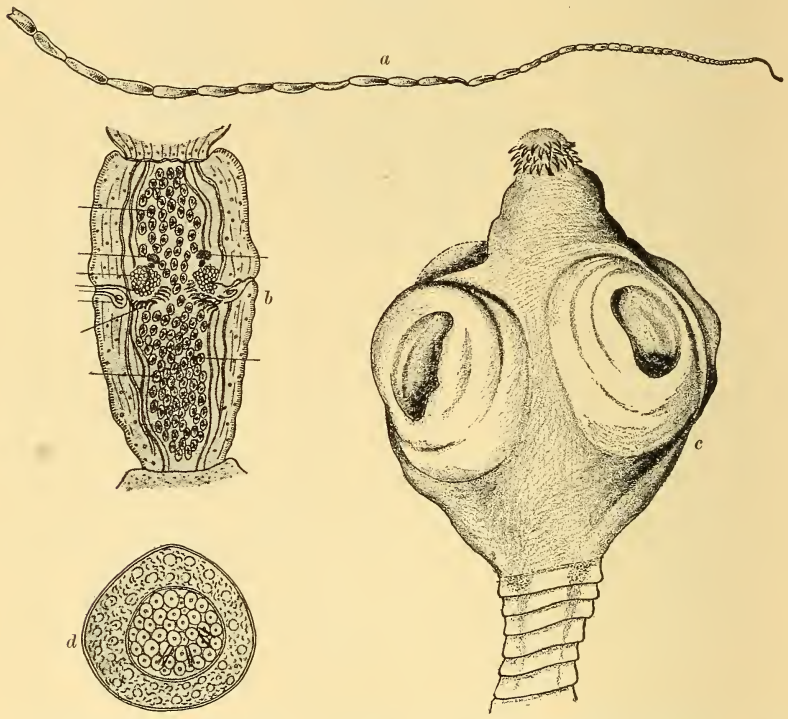
[1] Grassi, Centralbl. f. Bakt. u. Parasit., 1887, vol. i. p. 97. Grassi u. Calandruccio, Ibid., 1887, vol. ii. p. 282. Comini, Ibid., p. 27. Bilharz, cited by Leuckart. C. W. Stiles, New York Med. Jour., Nov. 7. 1903.

[2] Leidy and Parona, cited by Leuckart.

[3] C. W. Stiles, Amer. Med., 1902, vol. v. p. 65.

half of segment; uterus forms egg capsules, each containing from 8 to 20 eggs, eggs globular, 43–50 μ in diameter. The ova contain embryos already armed with hooklets (Stiles). In diagnosis Stiles suggests that search be made in the feces for the peculiar elongated elliptical tapeworm segments (Fig. 63). Microscopical examination of the feces for eggs is less certain than in cases of infection with Tænia saginata, Tænia solium, or Dibothriocephalus latus, since Dipylidium is much smaller and less prolific than any of these three forms.[1]

Fig. 63.



a, Dipylidium caninum (taken from Stiles); *b*, gravid segment (after Diamare); *c*, head, showing four rows of rose-thorn hooks on the rostellum and four unarmed suckers (Stiles); *d*, egg, showing six hooks of the embryo (Stiles).

Tænia africana, v. Linstow.[2] This parasite has been found in two instances, in the case of two native soldiers at Nyasa Lake. Like the scolex of Tænia saginata that of the present species is devoid of hooklets. Its length is about 1.4 m.; the number of segments about 600. They are all much broader than long. The uterus

[1] A. Hoffmann, Jahresb. f. Kinderheilk., 1887, vol. xxvi. Hefte 3 u. 4. Krüger, St. Petersburg. med. Woch., 1887, vol. xii. p. 341. Brandt. Centralbl. f. Bakt. u. Parasit., 1889, vol. v. p. 99.

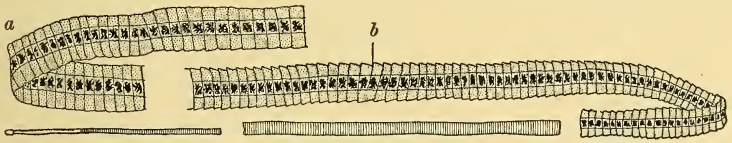
[2] v. Linstow, Centralbl. f. Bakt. u. Parasit., 1900, vol. xxviii. p. 485.

consists of a main portion running fore and aft, from which from 15 to 24 side branches issue, which do not branch dichotomously and are so closely packed that they cannot be recognized with the naked eye.

Tænia Madagascariensis (Grenet).—This parasite has been found in Madagascar, in Mauritius, in Bangkok, and in a Demarara Indian. The worm attains a length of from 25 to 30 cm. and is composed of from 500 to 600 trapezoid segments. The rostellum is surrounded by a double row of minute hooklets. The suckers are round and quite large. Blanchard suggests that the cockroach may be its intermediary host.

Bothriocephalus latus, Bremser : *syn.*, Tænia lata (Linné) ; Dibothrium latum (Rudophi) (Figs. 64–67). This worm is usually 5–10 m. long and of a reddish-gray color. Longer specimens, however, may also be encountered. In Wilson's case 82 feet of segments were obtained from two worms, so that the length of each,

Fig. 64.



Bothriocephalus latus: *A*, *B*, twin segments. (Wilson.)

supposing both to have been of the same size, must have been more than 40 feet. The head is almond-shaped and upon its flat surfaces two distinct grooves can be discerned, which probably act as suckers. It measures 2–3 mm. in length by 1 mm. in breadth. The neck is very short and passes at once into the body segments. Adjacent segments can often be distinguished only by means of the recurrence of the sexual apparatus, which appears regularly in spite of the imperfect individualization of the segments. The ripe segments are almost square in form, with the genital apparatus opening in the median line. The fully developed segments measure 2.5–4.5 mm. in length by 8–14 mm. in breadth. The total number of segments may far exceed 3000. The frequent occurrence of imperfect and abortive types of twin segments may be considered an almost distinctive feature of the bothriocephalus family (Wilson). The uterus presents 4 to 6 convolutions on each side, which become especially distinct when the segments are placed in water or are exposed to the air. A rosette-like appearance is then noted, which is quite characteristic. The rosette deepens in color in proportion to the number of ova which the uterus contains, and toward the tail of the parasite, from the segments of which many or all the eggs have

been discharged, the rosette tends to become light in color, and may indeed appear whiter than the surrounding parenchyma. The eggs (Fig. 65) are oval, 0.06–0.07 mm. long and about 0.045 mm. broad; they are enclosed in a brown envelope, at the anterior end of which a little lid can be recognized. Their contents consist of protoplasmic spherules, all of about the same size, which are lighter in color in the centre than at the periphery. In infested individuals they are constantly found in the stools.

The larvæ have been found in various fresh-water fishes, such as the perch, the ling, the turbot, in various members of the trout family, but they are most commonly encountered in the pike. It is thus readily understood why the parasite is most common in lake regions, as in Switzerland, northern Russia, southern Scandinavia,

Fig. 65.

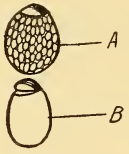
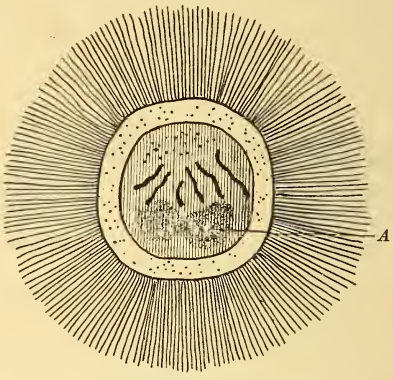


Fig. 66.

Plerocercoid. (Braun.)

Fig. 67.



Embryo with cilia and hooklets: *A*, bundles of muscle-fibres. (Leuckart and Braun.)

and northern Italy. It is seldom seen in middle Germany, but is so common in Ireland that Cobbold named it the Irish tapeworm. Outside of Europe it is most common in Japan. In the United States a few imported cases have been observed by Walker and Leidy, Packard, Hageestam, Riesman, Stengel, McFarland, and Wilson.

Multiple infection has been repeatedly observed. Böttcher notes a case in which 100 worms were found; Roux and Eichhorst both speak of cases with 90, Heller of one with 38, and in Wilson's case 2 were undoubtedly present. When more than one occurs, the growth of the individual is impeded, and small specimens are then usually seen (3–5 feet or more). Clinically the parasite is of especial interest as its presence in a certain percentage of cases is associated with the clinical picture of a pernicious anæmia; in others, however, no deleterious effect upon the red corpuscles is noted, although several worms may be present in the intestinal tract.

Besides in man, the worm has been encountered in the dog, cat, the seal, and in some water birds. The ovum after being discharged in the feces, during a variable period of incubation in the water develops into the onchosphæra, a ciliated larva with 6 hooklets (Fig. 67). The larva is then liberated from the ovum by passing through the lidded end, and by means of its cilia moves rapidly through the water. If not eaten by fish, it dies; otherwise it develops into the bothriocephalus measle, the plerocercoid (Fig. 66), which has both head and tail. Infection of man then occurs when such fish are eaten either raw or but partly cooked. In man the cysticercus stage has not been observed.[1]

Krabbea grandis, Blanchard. This parasite has been observed in only one instance—in Japan. It is said to resemble certain bothriocephali which are found in seals. The genital organs are double in each segment. The vulva and uterus open ventrally. The worm attains a length of 10 m. with a breadth of 2 cm.

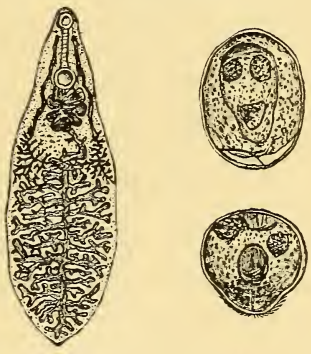
Trematodes.—The various forms of distoma which belong to this order are essentially hepatic parasites, and rarely occur in the feces.

Distoma hepaticum, Abildgaard: *syn.*, Fasciola hepatica (Linné) (Fig. 68). This, the most common liver-fluke, is 28 mm. long and

Fig. 68.

Distoma hepaticum. (Leuckart.)

Fig. 69.



Distoma lanceolatum (× 8) and eggs. (v. Jaksch.)

12 mm. broad; it is formed like a leaf. The leaf is provided with a sucker, and a second sucker may be found at its ventral surface. Between the two the genital opening is located, leading into a skein-shaped uterus. The eggs are oval, measuring 0.13 mm. in length and 0.08 mm. in breadth, the anterior end being provided with a lid; their color is brown. In the United States the organism is practi-

[1] Schaumann, Zur Kenntniss d. sogenannten Bothriocephalus-Anaemie, Berlin, 1894. Schauman u. Tallqvist, "Ueber d. blutkörperchenauflösenden Eigenschaften d. breiten Bandwurms," Deutsch. med. Woch., 1898, p. 312. Runeberg, Deutsch. Arch. f. klin. Med., 1887, vol. xli. p. 304. Askanazy, Zeit. f. klin. Med., 1895, vol. xxvii. p. 492. R. N. Wilson, "Bothriocephalus, Report of a Case of Double Infection." Am. Jour. Med. Sci., 1902, vol. cxxiv. p. 262.

cally unknown, while in Germany it is most common in sheep. In the human being it is rare in both countries. It occurs in cattle, sheep, swine, cats, rabbits, etc. Infection occurs through a small snail, the Linnæus minutus, which is found, in Germany especially, upon watercress.[1]

Distoma lanceolatum, Mehlis, has been found in only five cases, all of which occurred in Germany (Fig. 69). It is much smaller than Distoma hepaticum, measuring 8 to 9 mm. in length, by 2 to 3.3 mm. in breadth. It is lancet-shaped, tapering toward the head-end, but otherwise closely resembles the above parasite. The ova are 0.04 mm. long, 0.03 mm. broad, and contain fully developed embryos. In cattle, sheep, and hogs the organism is quite common.[2]

Distoma Buski, Lancaster: *syn.*, Distoma rhatonisii (Poirier); Distoma cranum (Busk). The parasite has been observed in China, Sumatra, the Straits Settlements, Assam, and India. It is the largest distoma occurring in man, measuring over an inch in length. It probably inhabits the upper portion of the intestine and may give rise to attacks of recurring diarrhœa and other signs of intestinal irritation. Infection probably occurs through certain fishes and oysters.[3]

Distoma sibiricum, Winigradoff: *syn.*, Distoma felinum (Rivolta). This parasite was found in Tomsk, by Winigradoff, in eight autopsies out of one hundred and twenty-four. Askanazy also reports two cases of infection from eastern Prussia, in which the eggs were found in the stools. In one of the cases, which came to section, more than one hundred organisms were found in the biliary passages. Its length may reach 13 mm. The ova are 0.026 to 0.038 mm. long and 0.010 to 0.022 mm. broad. The intestine is simple and extends to the posterior extremity of the body. Its surface is smooth.[4]

Distoma spatulatum, Leuckart: *syn.*, Distoma sinense (Cobbold); Distoma endemicum (Balz); Distoma japonicum (Blanchard). It has been observed in India, Mauritius, Corea, Formosa, China, Tonkin, and Japan, and it appears that in the two last-named countries it is quite common. It inhabits the biliary passages and gall-bladder. It is distinctly pathogenic. The ova may be found in the stools. The parasite possibly also occurs in cats. The intermediary host is not definitely known; it may be some fresh-water mollusc. It is about 11.75 mm. long and 2 to 2.75 mm. broad. The living parasite is of a reddish color and translucent, so that it is possible to distinguish all its interior organs. The ova measure 0.028 to 0.030 mm. in length by 0.016 to 0.017 mm. in breadth, and are enclosed in a colorless envelope.[5]

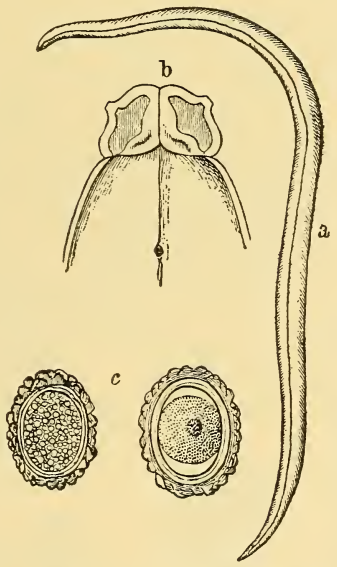
[1] C. W. Stiles, Jour. Comp. Med. and Vet. Arch., 1894, vol. xv., and 1895, vol. xvi. Huber, Trematoden. Bibliog. d. klin. Helminthol., Hefte 7 u. 8, p. 283.
[2] Leuckart, loc. cit., p. 137.
[3] Poirier, Centralbl. f. Bakt. u. Parasit., 1888, vol. ii. p. 186.
[4] Winigradoff, cited by Braun, Centralbl. f. Bakt. u. Parasit., 1894, vol. xv. p. 602.
[5] Blanchard, loc. cit.

Other parasites belonging to this order are **Distoma conjunctum** (Cobbold), **Distoma heterophyes** (v. Siebold), and **Amphistomum hominis** (Lewis and MacConell). The last-named appears to be common in elephants and has been encountered in natives of Assam, in two Indians in Calcutta, and in an East Indian immigrant in British Guiana. It is quite small, measuring from 5 to 8 mm. in length by 3 to 4 mm. in breadth and is characterized by the large size of its posterior suckers.

Distoma heterophyes is the smallest distoma, so far as we know, which is found in man. It occurs in Egypt and is thought to be innocuous.

Distoma conjunctum was discovered in an East Indian. Its surface is covered with minute spicules. It is not of much pathological importance.

FIG. 70.



Ascaris lumbricoides. (v. JAKSCH.)

a, worm, half natural size; *b*, head slightly magnified; *c*, eggs. (Eye-piece I., objective 8 A, Reichert.)

Distoma hæmatobium and **Distoma pulmonale** are described in the sections on the Blood and the Sputum, respectively.

Annelides.—The annelides are very common intestinal parasites, and of these especially the *nematodes.*

Ascaris lumbricoides, Linné (Fig. 70), is the cylindrically shaped worm so commonly seen in children and in the insane. The head consists of three projections or lips, which are provided with suckers and fine teeth. The male measures about 215 mm., the female about 400 mm. in length. The tail-end of the male is rolled up on its

ventral surface like a hook, and is provided with papillæ. The genital aperture of the female is situated directly behind the anterior third of the body. The eggs are yellowish brown in color, almost round, and measure 0.06 mm. by 0.07 mm. in size; they are surrounded by an irregular albuminous envelope, which is covered by a tough shell; the contents are coarsely granular.

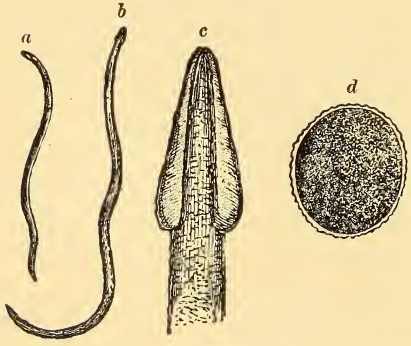
Ascaris lumbricoides is found in all countries, and also infests the pig and the ox. Its presence may occasion severe nervous symptoms, but fortunately this is rarely the case.[1]

Ascaris mystax, Zeder: *syn.*, Ascaris marginata (Rudolphi); Ascaris alata (Bellingham) (Fig. 71). This worm is smaller and thinner than Ascaris lumbricoides, but otherwise very similar. The head is pointed and provided with wing-like projections, which constitute the main point of difference between the two. The male measures 45 to 60 mm. in length, the female 110 to 120 mm. Its ova are round, larger than those of Ascaris lumbricoides, and enclosed in a membrane which is covered with numerous small depressions. The worm is common in dogs and cats, but very rare in man.[2]

Ascaris maritima, Leuckart, also belongs to this class. It has been observed in only one case—in Greenland.

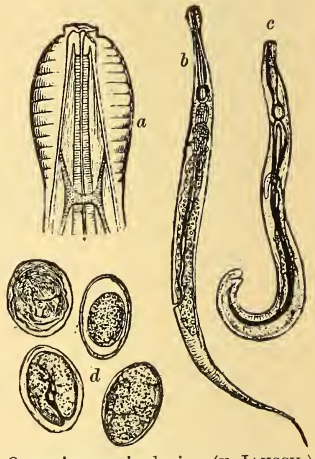
Oxyuris vermicularis, Bremser: *syn.*, Ascaris vermicularis (Linné); Ascaris græcorum (Pallas) (Fig. 72). The male is 4 mm., the female 10 mm. long. At the head three lip-like projections with lateral cuticular thickenings may be seen. The tail of the male is provided with six pairs of papillæ, and

FIG. 71.



Ascaris mystax. (V. JAKSCH.)
a, male; *b*, female; *c*, head; *d*, egg.

FIG. 72.



Oxyuris vermicularis. (V. JAKSCH.)
a, head; *b*, male; *c*, female; *d*, eggs.

[1] Lutz, Centralbl. f. Bakt. u. Parasit., 1888, vol. iii. pp. 553, 584, 616. Hogg, Brit. Med. Jour., 1888, p. 121. Kartulis, Centralbl. f. Bakt. u. Parasit., vol. l. p. 65.

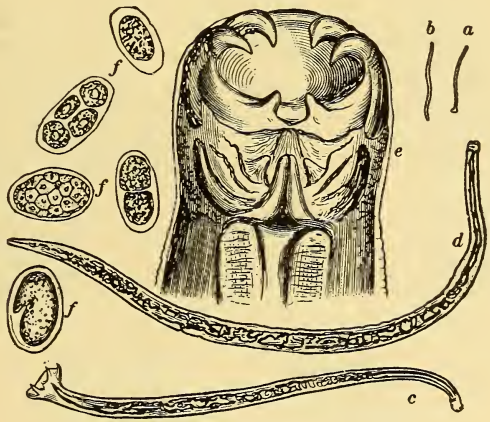
[2] K. A. Rudolphi, Arch. f. Zool. u. Zoot., 1803, vol. iii. Pt. 2, p. 1. Idem, Entozoorum s. vermium intestinal. historia naturalis, Amstelaedami, ii. 2.

the female with two uteri. The eggs are 0.05 by 0.02 to 0.03 mm. in size, and covered with a membrane showing a double or triple contour; in the interior, which is coarsely granular, the embryos are contained.

The female worm lives in the cæcum, but after impregnation travels downward to the rectum. Here it causes most annoying symptoms, which are especially distressing at night, when the organism emerges from the anus. In doubtful cases of pruritus ani et vulvæ an examination of the feces should be made for this parasite. The ova themselves do not occur in the feces.[1]

Anchylostomum duodenale (Dubini): *syn.*, Anchylostoma duodenale (Dubini); Strongylus quadridentatus (v. Siebold), Dochmius anchylostomum (Molin); Sclerastoma duodenale (Cobbold); Strongylus duodenalis (Schneider); Dochmius duodenale (Leuckart); Uncinaria duodenalis (Roilliet) (Fig. 73). This organism belongs

FIG. 73.



Anchylostomum duodenale. (v. JAKSCH.)
a, male, natural size; *b*, female, natural size; *c*, male, magnified; *d*, female, magnified; *e*, head (eye-piece II., objective C, Zeiss); *f*, eggs.

to the family *Strongyloides*, and is one of the most dangerous parasites met with in the human being. It has been found in Italy, Germany, Switzerland, Belgium, Egypt, and in the West Indies (Jamaica). In the United States a few imported cases of anchylostomiasis have also been met with, but the impression has been general that the disease was quite rare. C. W. Stiles, however, has recently shown that a distinct species of the hook-worm exists in this country as also in the West Indies, viz., in Cuba and Porto Rico, the *Uncinaria Americana*, and that in the sand regions of the South infection with this parasite is common, especially among the lower classes of negroes in many of whom infection probably occurs in consequence of the habit of dirt-eating.

[1] Lutz, loc. cit.

From a pathological standpoint the parasite is of special interest, as its presence may give rise to severe and often fatal anæmia. Griesinger was the first to point out that the so-called Egyptian chlorosis is produced by this organism. Subsequently it was shown that the same parasite was responsible for the anæmia which developed among the workers on the St. Gotthard tunnel, and which was common among brickmakers in certain districts in Germany. In this country the anæmia of the dirt-eaters has long been known in the South, and has been generally attributed to the peculiar habit. Its real cause is now manifest. While infection no doubt generally takes place by a direct transference of the embryos with dirty hands, it may certainly also occur through contaminanted drinking-water.

Outside of man the parasite is not uncommon in dogs, cattle, and sheep.

FIG. 74.

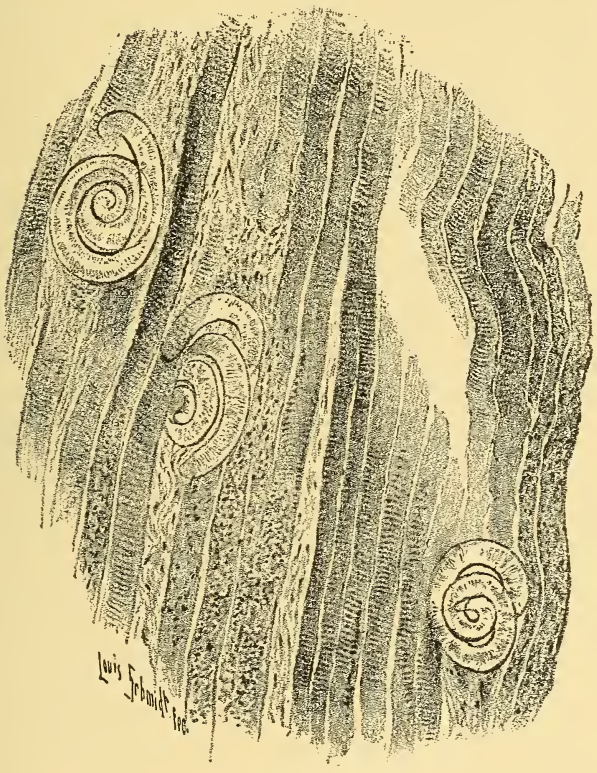
Eggs of Uncinaria americana, in different stages of development (personal observation). Magnified about 300.

The male is 6 to 11.5 mm. long, the female 10 to 18 mm. The head, which tapers somewhat, is turned toward the back ; the mouth capsule is hollowed out and surrounded by 4 teeth ;[1] the tail of the male forms a 3-lobed bursa, while that of the female tapers conically ; the genital opening is behind the middle of the body. Its eggs have an oval form and a smooth surface, measuring from 0.05 to 0.06 by 0.03 to 0.04 mm. In their interior two or three segmenting bodies are found, which rapidly develop outside of the human body, so that after twenty-four to forty-eight hours embryos may be found in the same feces in which the eggs were observed,

[1] The American species has only one dorsal, conical tooth, which projects prominently into the buccal cavity (Stiles).

or fully developed ova may be found after allowing the feces to stand for only a few hours (Fig. 74). When allowed to dry, the young parasites become encysted, but after remaining so even for from one to two weeks they are capable of infection. A second host for its cycle of development is, according to Leichtenstern, not necessary.[1]

Fig. 75.



Trichina spiralis in muscle.

The habitat of the adult worm is the jejunum. It is rarely found in the feces. Its eggs, however, are common, and should be looked for in every case of anæmia the cause of which is not manifest, especially in miners, tunnel-workers, brickmakers, dirt-eaters, etc. Any specimen of fecal material will answer as a rule, but it is best to procure a thin stool, as after a purge. It is then merely neces-

[1] Leichtenstern, Centralbl. f. klin. Med., 1885, vol. vi. p. 195; Deutsch. med. Woch., 1885, vol. xi.; 1886, vol. xii.; 1887, vol. xiii. Lutz, Volkmann's Sammlung, 1885, Nos. 255 and 256. American cases: C. W. Stiles, "The Significance of the Recent American Cases of Hook-worm Disease," 18th Annual Report Bureau Animal Industry, 1901. H. F. Harris, Amer. Med., Nov. 15, 1902, p. 776. A. J. Smith, Am. Jour. Med. Sci., 1903, vol. cxxvi. p. 768. C. F. Craig, Ibid., p. 798.

sary to mount a small drop on a slide and to examine the covered specimen with a low power; a Bausch & Lomb $\frac{2}{3}$ is quite sufficient. A mental picture of the size of the eggs should be made, for I have known it to occur that an observer saw the eggs, but did not recognize them as such. Once seen, they are easily recognized again.

Trichocephalus hominis, Schwank: *syn.*, Trichocephalus dispar (Rudolphi); mastigodes (Zeder); trichuris (Büttner). This parasite, which belongs to the family *Trichotrachelides*, is formed like a whip, the last-end being the head-end, while the tail-end is very much thicker. The male measures 46 mm. and the female 50 mm. in length. The eggs are brownish in color, measuring 0.05 by 0.06 mm. in size, and present a doubly contoured shell, with a depression at each end, closed by a lid. The contents are coarsely granular. The organism is said to be the most widely distributed intestinal parasite, occurring in Europe, North America, Asia, Africa, and Australia. Its habitat is the cæcum. The living worm is only rarely found in the feces.[1]

Trichina spiralis (Owen) (Fig. 75) is rarely found in the feces. The male measures 1.5 mm. in length, and is provided with four papillæ between the conical lips. The female is 3 mm. long. The uterus is situated nearer the head than the ovary, which opens into it. Fertilization occurs in the intestinal canal. The eggs develop into embyros in the uterus, emerge from this, and penetrate the intestinal walls, whence they are carried by the blood-current to the muscles. Trichinosis is far less common in the United States than in Europe.[2] The diagnosis of sporadic cases has been greatly facilitated by the discovery of Brown that eosinophilia, often of high grade, is practically of constant occurrence during the acute stage of the disease (see page 102).

Strongyloides intestinalis (Bavay): *syn.*, Anguillula intestinalis (Bavay); Anguillula stercoralis (Bavay); Rhabditis stercoralis (Bavay); Leptodera stercoralis (Bavay, Cobbold), Leptodera intestinalis (Bavay, Cobbold); Strongyloides intestinalis (Bavay, Grassi); Pseudo-rhabditis stercoralis (Bavay, Perroncito); Rhabdonema strongyloides (Leuckhart); Rhabdonema intestinale (Bavay, Blanchard).

In the feces of patients infested with the parasite in question the eggs of the mother-worm are only rarely found, and the worm itself probably never appears unless an anthelmintic has been administered and active catharsis established. Instead we find embryos (rhabditic form) measuring about 0.33 by 0.022 mm. in size. If the stools are kept, uncovered, at a temperature of about 37° C., their larvæ undergo development and reach full growth and sexual differentiation in almost five days. The length of the full-grown female is about 1 mm.; its breadth about 0.04 mm. The body is

[1] Ermi, Berlin. klin. Woch., 1886, vol. xxiii. p. 614. [2] Leuckart, loc. cit.

cylindrical, slightly diminishing in size anteriorly and tapering to a sharp point posteriorly. When the worm retracts forcibly, slight transverse furrows may be seen. The mouth possessed distinct lips and is continnous with a triangular œsophagus, which beyond a constriction dilates again into a second ovoid enlargement. The intestine which follows ends in a little protrusion on one side of the body near the base of the tail. A little below the middle of the body, and on the ventral side, is the vulva, which leads to the uterus, extending from the intestinal ventricle to a point near the anus. Here the eggs may be massed in varying numbers. Sometimes the young have actually broken the shell of their eggs and may be seen free in the uterus; but more commonly the ova, on deposition, contain well-formed motile embryos (filariform brood). The male is about one-fifth smaller than the female. The testicle ends at the base of the tail in two small horn-like spicules with tapering ends, which are curved inward. These spicules contain canals; they are of equal size and situated symmetrically on a transverse plan. The tail is coiled in the same direction as the spicules, and is half as long as that of the female.

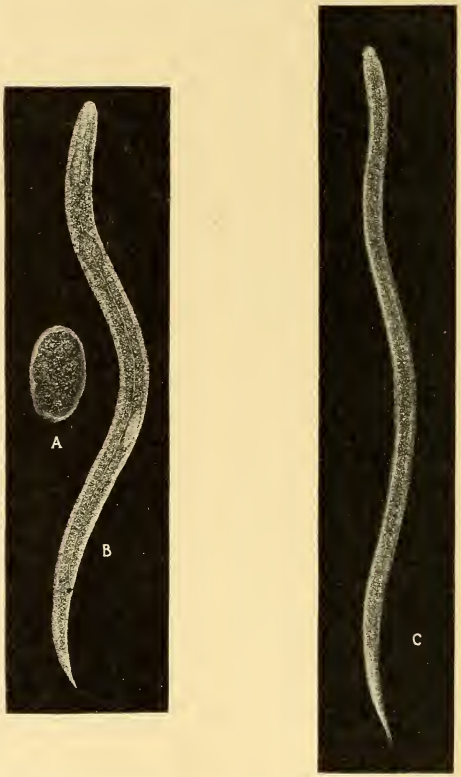
The sexually mature and differentiated forms just described represent the *Anguillula stercoralis* of Bavay. They represent an intermediate generation, developing outside of the body, which forms a link in the chain of development of the mother-worm, the *Anguillula intestinalis* (Leuckart).

Ordinarily infection takes place through the larvæ of the sexually differentiated form. These filariform embryos are longer than the rhabditiform brood of Anguillula intestinalis (Fig. 76). They are provided with a cylindrical œsophagus descending down to about the middle of the body, and a tail, which instead of terminating in a fine point, is apparently truncated at its extremity. On maturation they give rise to the Anguillula intestinalis, which is encountered throughout the upper gastro-intestinal tract, especially in the lower part of the duodenum and the upper part of the jejunum, though occasionally they have also been found throughout the entire jejunum and in the upper part of the ileum. On several occasions they have been found in the stomach.

Anguillula intestinalis, viz., the parasitic mother-worm, is, according to Rovelli, parthenogenetic, while Leuckhart expressed the opinion that it might be hermaphroditic. Its length is abont 2.20 mm., and its average breadth 0.03 mm. The body tapers a little anteriorly, and terminates posteriorly in a conical tail, the extremity of which is appreciably rounded and even a trifle dilated. The mouth is without horny armature, and shows 3 small lips. It opens into a cylindrical œsophagus, which occupies about one-fourth of the length of the animal, and shows neither swellings nor striations. The intestine extends nearly to the posterior extremity of the body,

but is almost invisible in the middle part owing to the presence of a large elongated ovary. The vulva is situated in the posterior third of the animal, and the uterus contains usually 5 or 6 rather elongated ova. The anus is situated toward the base of the tail. The eggs are of a yellowish-green color, rather opaque, and apparently finely granular (Bavay); in their general appearance they resemble those of the Uncinaria (Fig. 76, A.).

FIG. 76.



A, egg of Strongyloides intestinalis (parasitic mother worm); B, rhabditiform embryo; C, filariform embryo, derived by direct transformation, from a rhabditiform embryo. (Taken from Thayer.)

While infection originally takes place through the filariform larvæ of Anguillula stercoralis, an auto-infection with the larvæ may also occur without the intervention of the sexually differentiated forms, by a direct transformation from the rhabditiform embryos of the parasitic mother-animal, and there is evidence to show that this latter cycle is indeed the more common. There is no evidence to show that the sexually mature intermediate generation ever develops in the intestinal tract during life.

The time elapsing between infection with the filariform larvæ and the appearance of rhabditiform embryos in the stools is about seventeen days.

The parasite is the recognized cause of the so-called Cochin-China diarrhœa, and is of further interest from its resemblance to Anchylostoma duodenale, with which it is not infrequently found associated Excepting in very rare instances, it does not cause intestinal ulceration, and it is supposed that the injurious effects of the parasite are purely mechanical. It is possible, however, that these may also be owing to the irritating action of its excretory products. The clinical manifestations of the disease are mainly those of a chronic diarrhœa and a comparatively mild anæmia. There are usually 3 or 4 pasty stools a day.

The organism was first discovered in individuals who had contracted severe diarrhœa in Cochin-China. Grassi and Parona later found the worm in Italy, and at the building of the St. Gotthard tunnel it was frequently seen in association with the Anchylostoma. Thayer was the first to find it in the United States, and it is interesting to note that two of his three cases must have become infested in either Maryland or Virginia. The third case may have originated in Austria; in it the anguillula was associated with amœbæ and the Trichomonas intestinalis; it ended fatally, being complicated by liver abscess. Since then additional cases have been reported in the United States by Moore and Price.

Other cases have been observed in Belgium, Holland, Martinique, Brazil, Sicily, the Dutch Indies, Egypt, Germany, Spain, and the Philippine Islands.

LITERATURE.—Grassi, Centralbl. f. Bakt. u. Parasit., 1887, vol. ii. p. 413. Leichtenstern, Deutsch. med. Woch., 1898, p. 118. Perroncito, Arch. p. l. sci. med., 1881, No. 2. Compt. rend. de l' acad. des sci., 1882, No. 1. Teissier, Ibid., vol. cxxi. p. 171. Bavay, Ibid., 1876, vol. lxxxiii. p. 694; Ibid., 1877, vol. lxxxiv. p. 266. Normand, Ibid., 1876, p. 316. W. S. Thayer Jour. of Exper. Med., 1901, vol. vi. No. 1 (full literature to 1901). M. L. Price, Jour. Am. Med. Assoc., Sept. 12, 1903 (literature to date since Thayer's paper).

Insecta.—As the larvæ of the various insects met with in the feces have been very little studied, they will not be considered at this place; they are apparently of no clinical importance.

Vegetable Parasites.—Among the pathogenic vegetable parasites the bacillus of cholera, of typhoid fever, and of tuberculosis, as well as the bacilli of Booker, the Bacillus coli communis, the Bacillus pyocyaneus, the Bacillus lactis aërogenes, the bacillus of Shiga, and the Proteus vulgaris, deserve especial consideration.

The Comma-bacillus.—As early as 1848 certain "vibrios" were observed in abundance in the stools of *cholera* patients by Virchow, and in 1849 by Pouchet, Britton, and Swayne, no importance, however, being attached to their presence at the time.

The first accurate and detailed studies of the organism found in cholera stools were made in 1883 by the members of the French and German commissions sent to Egypt to investigate the nature of the dreaded disease. The results of their work were first published by Koch in his report to the Berlin Sanitary Office in 1883, and in 1884 by Strauss, Roux, Nocard, and Thuillier.

The clinical recognition of cholera Asiatica has now become a simple matter since Pfeiffer has demonstrated that the blood-serum of cholera patients possesses the property of causing arrest of motility and agglutination of the specific bacilli. Ordinary bouillon-cultures, however, can usually not be employed, as particles of the film when broken up may easily be mistaken for agglutinated bacilli. It is best in every case to make use of agar-cultures sixteen to twenty-four hours old, and to prepare emulsions in bouillon or normal salt solution as occasion requires. The emulsion, moreover, should always be examined microscopically before use, so as to insure the absence of any conglomerations of bacilli. The blood is then diluted in the proportion of 1 : 10 or 1 : 15. If the test-tube method is employed, the tubes should be kept in the incubator (37° C.) for only one or two hours. Upon the slide the reaction is obtained in from five to twenty minutes. If no distinct agglutination is observed at the end of one hour, the diagnosis of cholera is rendered improbable. Dried blood retains its agglutinating properties for a considerable length of time, and may also be used for examination.

The comma-bacillus is a slightly arched or half-moon-shaped little rod, and is somewhat shorter than the tubercle bacillus (Plate XV., Fig. 1). Occasionally two are placed end to end with their convexities in opposite directions, thus presenting the appearance of the letter S. They are provided with flagella. Koch detected these bacilli in the intestinal contents and feces, but rarely in the vomited matter, in Asiatic cholera only. In the stools they at times occur in such numbers as to constitute pure cultures. In plate-cultures kept at a temperature of 22° C. white colonies with serrated borders may be observed after twenty-four hours. The color of such a colony is slightly yellow or rose red, its central portion gradually assuming a deeper tint, and finally becoming liquefied. Upon agar-plates the bacilli form a grayish-yellow, irregular, slimy coating, but do not liquefy the culture-medium. In stab-cultures, after twenty-four hours, a whitish color may be observed along the line of the stab ; around this there is formed a funnel-shaped depression, which gradually increases in size and apparently contains a bubble of gas. The upper portion of the culture-medium at the same time becomes liquefied, while the lower portion remains solid for days. In a suspended drop spirochætæ-like spirals are observed at the margins, which often present as many as twenty distinct arches.[1]

[1] R. Koch, Berlin. klin. Woch., 1884, vol. xxi. pp. 477, 493, 509.

PLATE XV.

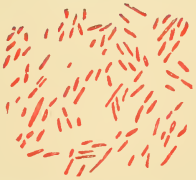
FIG. 1.

Spirillum of Asiatic Cholera. Impression Cover-slip from a Colony Thirty-four Hours Old. (Abbott.)

FIG. 2.

Bacillus of Finkler and Prior. (Cornil and Babes.)

FIG. 3.



Bacillus of Typhoid Fever from a Culture Twenty-four Hours Old, on Agar-agar. (Abbott.)

Closely related to Koch's comma-bacillus is the *bacillus of Finkler and Prior*, discovered in 1884 and 1885 (Plate XV., Fig. 2). This is, however, readily distinguished from the former by the following characteristics: it is larger and thicker than the comma-bacillus; the colonies on gelatin plate-cultures show equally round and sharp-edged forms, which present a granular appearance under a low or medium power, and are usually of a brown color. The organism liquefies gelatin very rapidly, a penetrating, excessively fetid odor being developed at the same time. In stab-cultures the bacillus of cholera Asiatica forms a funnel-shaped depression, while the bacillus of Finkler and Prior forms a stocking-like depression.[1]

In this connection the *green bacillus of Le Sage*, discovered in certain forms of infantile diarrhœa, must briefly be referred to, the stools, as has been mentioned, being of a grass-green color. The production of this pigment in cultures is one of the characteristics of the organism; when injected into the intestines of animals it is said to produce diarrhœa and a catarrhal inflammation of the mucous membrane.

Booker[2] has described nine different bacilli as occurring in cases of infantile diarrhœa. Seven of these closely resemble the Bacillus coli communis. Bacillus "A" is a bacillus with rounded ends, measuring from 3 μ to 4 μ in length by 0.7 μ in breadth. It is motile and liquefying. Colonies on agar and potato present a dirty-brown color.

The **typhoid bacillus**, discovered by Eberth[3] in 1880 in the abdominal organs of patients dead with typhoid fever, is unfortunately not so readily recognized in the feces as the organisms just described. This is owing to the intimate relation which apparently exists between the bacillus in question and the Bacillus coli communis, with which it has many properties in common. A few years ago Elsner suggested a method which, it was hoped, would effectually overcome this difficulty, and in the hands of numerous observers good results were obtained. Widal's agglutination test, however, which was almost simultaneously introduced, diverted attention from the study of the feces, and Elsner's work has practically been forgotten.

In the meantime Widal's test has been carefully investigated, and although the reaction must unquestionably be considered as a specific reaction of typhoid fever, its value in diagnosis is neverthe-

[1] Finkler, Deutsch. med. Woch., Tageblatt der Naturforscherversammlung, 1884, vol. x. p. 36, and 1885, p. 438. Finkler u. Prior, Ergänzungshefte z. Centralbl. f. allg. Gesundheitspflege, 1885, vol. i.

[2] W. D. Booker, "A Bacteriological and Anatomical Study of the Summer Diarrhœas of Infants," Johns Hopkins Hosp. Rep., vol. vi.

[3] Eberth, Virchow's Archiv, 1881, vol. lxxxiii. p. 486.

less limited (see page 100). As a consequence, further attempts have been made to discover a method which will enable the general practitioner to establish definitely the diagnosis of typhoid fever at an early stage of the disease. Whether or not Elsner's method (v. i.) has been deservedly abandoned, further investigations will show. At the present time another procedure, which was suggested by Piorkowski, is attracting widespread attention, as it is claimed that with this method the diagnosis can be made within twenty-four hours.

Piorkowski's Method.[1]—The necessary culture-medium is prepared as follows: normal urine of a specific gravity of about 1.020 is allowed to stand until the reaction has become alkaline; it is then treated with 0.5 per cent. of peptone and 3.3 per cent. of gelatin, boiled for one hour, and filtered immediately into test-tubes without any further application of heat. The test-tubes are closed with cotton, sterilized for fifteen minutes in a steam sterilizer at 100° C., and resterilized after twenty-four hours for ten minutes.

To examine the feces, one tube is inoculated with 2 œsen of the fecal matter, which should be as fresh as possible. From this tube 4 œsen are transferred to a second tube, and a third is inoculated with from 6 to 8 œesen from the one preceding. Plates are finally prepared and kept at a temperature of 22° C., as the presence of so small an amount of gelatin does not permit of exposure to higher temperatures. After sixteen to twenty-four hours an examination is made with a low power. At the expiration of this time the colonies of the colon bacillus appear as round, yellowish-brown, and finely granular specks, with well-defined borders, while the typhoid colonies show a peculiar flagellate appearance, from two to four fine colorless radicles usually starting from a light, highly refractive central focus. After forty-eight hours the radicles have greatly extended, and after forty-eight to fifty-six hours the colonies are perfectly developed and present a picture which strongly suggests the appearance of radishes, minute interweaving branches being given off in every direction, while no difference can be observed at this time between typhoid and colon bacilli which have been grown for control in 10 per cent. normal or bouillon-gelatin.

Piorkowski claims that he has thus been able to demonstrate the presence of typhoid bacilli in infected drinking-water, and in the feces of typhoid fever patients at a time when a positive result could not yet be obtained with Widal's test. Recent reports bear out the claims of Piorkowski, and the method can hence be recommended in doubtful cases.[2]

[1] Piorkowski, "Ein einfaches Verfahren z. Sicherstellung d. Typhusdiagnose," Berlin. klin. Woch., 1899, p. 145.

[2] A. Schütze, "Ueber d. Nachweis v. Typhusbacillen in den Faeces," Zeit. f. klin. Med., vol. xxxviii. p. 39.

Elsner's Method.[1]—The culture-medium is prepared as follows: an aqueous extract of potato (500 grammes to the liter) is treated with 10 per cent. of gelatin and boiled. The solution is then treated with 2.4 to 3.2 c.c. of a one-tenth normal solution of sodium hydrate, in order to secure the necessary degree of acidity, and then filtered and sterilized.

When needed, a portion is placed in an Erlenmeyer flask and treated with 1 per cent. of potassium iodide. The mixture is inoculated with fecal material and the necessary plates prepared. Upon this medium only a few species of bacteria will grow, principally the Bacillus coli and the typhoid bacillus. After twenty-four hours the Bacillus coli colonies are already mature, while the typhoid colonies can scarcely be made out with a low power. After forty-eight hours, however, they appear as small, highly refractive, extremely fine, granular colonies, closely resembling drops of water, which can be readily distinguished from the large, much more granular, brownish colonies of the Bacterium coli. This difference is brought out particularly well if diluted plates have been prepared.

Brieger,[2] who carefully repeated the experiments of Elsner, states that typhoid bacilli are found in abundance in the stools so long as fever exists, but with approaching convalescence they diminish in number and ultimately disappear. If, notwithstanding the absence of fever, bacilli are found in notable numbers during convalescence, a relapse may be anticipated.

In pure cultures the typhoid bacilli present the following features: they occur in the form of rods of almost one-third the size of a red blood-corpuscle, or in threads composed of several rods joined end to end (Plate XV., Fig. 3). Their ends are rounded; their length is equivalent to about three times their breadth. They are actively motile and provided with polar as well as lateral flagella. They grow very readily on bouillon-peptone gelatin, and after twenty-four hours colonies begin to appear. When slightly magnified, these present a faintly yellowish color; macroscopically they are barely visible. When kept at a temperature of 37° C. the formation of spores may be observed, especially when the organism is grown on media colored with phloxin-red or benzopurpurin. Gelatin is not liquefied; the growth is white and delicate, both along the line of the stab and on the surface. Cultivation in glucose-bouillon, or glucose-agar, does not give rise to the formation of gas, but after twenty-four hours the entire fluid becomes turbid. Milk is rendered feebly acid, but is not coagulated. No indol reaction is obtained when the organism is grown on peptone-containing media. On potato a very faint, whitish, almost invisible growth takes place. When grown on gelatin or agar that has been colored with neutral

[1] Elsner, Zeit. f. Hyg. u. Infektionskrank., 1895, vol. xxi. p. 25.
[2] Brieger, Deutsch. med. Woch., 1895, vol. xxi. p. 835.

red, the typhoid bacillus causes no change in color. Absolute identification is possible by means of Pfeiffer's agglutination-test (see Widal's reaction).

In cases of *paratyphoid* infection the corresponding organism may be found in the feces (see Blood).

Tubercle bacilli, when present in the feces, are indicative of intestinal tuberculosis, providing they are observed upon repeated examination and there are clinical symptoms pointing to the bowels as the seat of the disease; otherwise they may be referable to swallowed sputa. They may be demonstrated as described in the chapter on Sputum.

The **Bacillus coli communis,**[1] while constantly present in normal feces, is described at this place, as modern investigations have shown that it may at times develop pathogenic properties. It has been found in the pus in cases of purulent perforating peritonitis, angiocholitis, pyelonephritis, etc., and, as indicated elsewhere, at times forms the nucleus of gall-stones. It occurs in the form of delicate or coarse rods, measuring about 0.4 μ in length, which manifest a certain degree of motility, due to the presence of one or two polar flagella. The organism is stained by the usual anilin dyes, and is decolorized by Gram's method. The colonies upon gelatin closely resemble those of the bacillus of typhoid fever, forming small whitish specks *in* the gelatin, and delicate films with serrated borders *upon* the same medium, which, moreover, is not liquefied. On potato the organism forms a brownish pellicle, while the growth of the typhoid bacillus is nearly transparent. As in the case of the cholera bacillus, the nitrosoindol reaction can be obtained when the organism is grown upon peptone-containing media. In solutions of glucose active fermentation takes place. Litmus milk is rendered acid and is coagulated. Important also is the behavior of the organism when grown on gelatin or agar that has been colored with neutral red; in contradistinction to the typhoid bacillus, the colon bacillus then causes an intense green fluorescence.

The **Bacterium lactis aerogenes** (Escherich) closely resembles the organism just described, and may also at times develop pathogenic properties. It was recently found in a case of pneumaturia and in one of idiopathic bacteriuria. It is seen quite constantly in the stools of sucklings, but may also be met with in those of adults. It occurs in the form of rather stout rods, which frequently lie in pairs, resembling diplococci. The organism is non-motile. Like the Bacillus coli communis, it is decolorized by Gram's method. In plate-cultures it forms a dense white film; in stab-cultures a chain of white colonies resembling beads is seen. In the latter, moreover, if the stab is closed, bubbles of gas will be seen to form, which rapidly increase in number and size. Milk is coagulated in large lumps in twenty-four hours; at the same time, the formation of

[1] Flügge, Die Microrganismen.

gas is much more intense than in the case of the Bacillus coli communis.

The **Bacillus pyocyaneus** has within recent years been isolated from the stools of dysenteric patients, and has been proved the cause of several epidemics. The organism in question is a small motile bacillus measuring from 1 μ to 2 μ in length by 0.3 μ to 0.5 μ in breadth. It sometimes occurs in short chains, but is usually single. It is stained with the common anilin dyes, and is decolorized with Gram's method. It grows on the usual culture-media, and liquefies gelatin. In 2 per cent. glucose-bouillon no fermentation takes place. Litmus-milk is curdled in about forty-eight hours. Some varieties produce indol. Most characteristic is the production of certain pigments, viz., pyocyanin and a fluorescent bluish-green pigment which is common to almost all varieties.[1]

Bacillus acidophilus, Moro.[2] This organism has recently been described by Moro as occurring in the stools of breast-fed infants, in which it normally prevails over all other forms; under pathological conditions, on the other hand, as also in the stools of children, which have been fed with cows' milk their number is found diminished, while the members of the coli-group enter into the foreground. Beyond the stools, the bacillus has been found in the outer portion of the secretory duct of the human mammary gland, in the milk, and the skin of the nipple and its immediate surroundings. It is apparently not pathogenic.

The organism occurs in the form of slight rods measuring 1.5 μ to 2 μ in length, by 0.6 μ to 0.9 μ in breadth. It is non-motile. It is not decolorized by Gram's method, but loses this property after from thirty-six hours to nine days. The best growths are obtained on beer wort bouillon and common bouillon when acidified with a mineral acid; the acidity of 10 c. c. of the medium may correspond to 10 c.c. of a decinormal solution of potassium hydrate. The optimum temperature is 37° C.; between 20° C. and 22° C. no growth occurs. On the various agar-slants imperfect development takes place; on potato the organism does not grow. It is an active acid-producer, but does not give rise to the formation of gas; with Escherich's stain it is colored blue.

Escherich's Stain.—This stain is now extensively used by pædiatrists in order to ascertain any deviations from the normal in the flora of the feces. Under strictly normal conditions the bacilli which are found in the stools of breast-fed children are thus nearly all colored blue (see above), while red bacilli are but little numerous. In the case of infants, on the other hand, which are fed exclusively on cows'

[1] A. J. Lartigau, "A Contribution to the Study of the Pathogenesis of the Bacillus Pyocyaneus," etc., Jour. Exper. Med., 1898, No. 6.

[2] Moro, "Ein Beitrag zur Kenntniss der normalen Darmbacterien des Säuglings," Jahrbuch f. Kinderheilk., vol. lii. Also: "Ueber die nach Gram färbbaren Bacillen d. Säuglingstuhles," Wien. klin. Woch., 1900, No. 5.

milk the red bacilli predominate, while in mixed feeding the blue enter into the foreground in about the proportion in which breast-milk is employed. The red bacilli belong to the coli-group. These further predominate, or may be found exclusively, if for any reason intestinal digestion is impaired. Staphylococci, streptococci, etc., when simultaneously present, are in either event stained blue. In staphylococcus enteritis the blue bacilli which normally exist in the stools of breast-fed infants are almost entirely replaced by staphylococci. At the beginning of the enteritis they are not numerous, but they increase during the progress of the disease, and finally disappear when the child recovers.

In staining, the following solutions are employed:

1. An aqueous solution of gentian-violet (5 : 200). This is boiled for one-half hour and is then filtered; it keeps for a long time.

2. A mixture containing 11 parts of absolute alcohol and 3 parts of oil of anilin.

(1) and (2) are mixed in the proportion of 8.5 : 1.5; the resulting solution keeps for from two to three weeks, but not longer.

3. A solution of iodo-potassic iodide containing 1 part of iodine and 2 parts of potassium iodide in 60 parts of water.

4. A mixture of equal parts of oil of anilin and xylol.

5. A concentrated alcoholic solution of fuchsin, diluted with an equal volume of absolute alcohol.

A bit of the stool is spread upon a slide in as thin a layer, as possible. After drying in the air the specimen is fixed by passing through the flame of a Bunsen burner. It is then stained for a few seconds with the mixture of (1) and (2), blotted, placed in the iodine solution for a few seconds, blotted again, decolorized with (4) until a notable extraction of color no longer occurs. It is washed with xylol, dried, and finally stained for a few seconds with the fuchsin solution, washed with water, blotted, and is then ready for examination.[1]

Proteus vulgaris, Hauser. This organism, while usually regarded as non-pathogenic, should be numbered among the bacteria which may at times develop pathogenic properties. Baginsky and Booker have frequently found it in the stools in cases of infantile summer diarrhœa. Escherich observed it at times in the meconium. Brudzinski examined the dyspeptic and fetid stools of a number of artificially fed infants in Escherich's clinic, and in all the cases found the proteus. Others have encountered it in inflammatory conditions of exposed surfaces, in appendicitis, in perforative peritonitis, and even in closed abscesses, either alone or in association with other bacteria (Welch). A mixed infection with the proteus and Löffler's bacillus has also been observed. The organism forms rods, measuring about 0.25 μ in diameter, while their length is

[1] Moro, loc. cit.

variable; at times a more roundish form is observed; at others rods measuring from 1.25 μ to 3.75 μ in length, or even long threads. They are readily stained, but are easily decolorized by alcohol or Gram's method. Most characteristic is their growth upon nutrient gelatin. At the temperature of the room little depressions will be observed after six to eight hours, which are surrounded by a narrow zone of bacilli from which a thin, wide film, provided with irregular projections, extends over the culture-medium. From this film islets become separated, which slowly extend over the gelatin and cause its liquefaction. The organism is motile. It decomposes urea and causes albuminous putrefaction. The nitroso-indol reaction is readily obtained in bouillon-cultures.[1] In boiled milk the organism grows well, while in fresh milk it develops only irregularly, and in acid milk no growth takes place at all.

Bacillus dysenteriæ, Shiga. This organism is now generally regarded as the specific cause of the common form of acute dysentery which prevails not only in the tropics, but also in the United States and Europe. It was discovered by Shiga in Japan, in 1897, and is identical with the organism obtained by Flexner and Strong in the Philippines and Porto Rico, by Vedder and Duval in the United States, and by Kruse in Germany. From the researches of Bassett and Duval it further appears that the same bacillus is also responsible for the common form of infantile summer diarrhœa which prevails in warm countries.

The bacillus in question is a short rod with rounded ends, which much resembles the typhoid bacillus and most members of the colon group. It is probably non-motile so far as active locomotion is concerned, but it is possessed of a high degree of molecular motion. It stains with the usual basic dyes and is decolorized by Gram's method.

Upon gelatin plates at room temperature there appear, after a few days, small round dots, which, magnified under low powers, are slightly yellow and finely granular. After a few days they increase in size; the middle portion of the colonies then appears darker under a low power, while the outer zone appears brighter and more seed-like. The superficial and deeper colonies show no marked variation. In stab-cultures of gelatin a whitish strand forms the whole length of the stab. The gelatin is not liquefied.

After twenty-four hours in the incubator single colonies upon slanted agar appear moist, bluish, and partially translucent. After two days they present a combination of a middle dark and a peripheral bright, sharply defined zone.

The growth on glycerin-agar is slightly more abundant than on ordinary agar. The organism grows on blood-serum without liquefying it.

[1] Flügge, loc. cit.

In the stab-cultures on glucose-agar there is formed along the whole line of the puncture a thick gray-white strand without the development of gas. Upon potato after twenty-four hours in the incubator there is hardly any perceptible growth, only the surface appears slightly shiny. After two days this changes to a yellow brown. In the course of a week the growth is heavier and of a deeper brown color. Bouillon cultures show after a day in the incubator a somewhat intense cloudiness, with a moderate precipitate. No pellicle is formed on the surface. No indol reaction is present. Litmus-milk after twenty-four hours appears reddish; otherwise, however, it undergoes no change. The milk never coagulates.

The bacillus is pathogenic for mice, rabbits, and guinea-pigs. It is agglutinated by the patient's blood-serum, and it is interesting to note that this reaction is obtained only with cases definitely known to have been infected with the micro-organism in question.

Isolation of Shiga's Bacillus from the Feces.—The fecal matter is collected on a sterile pad, or, still better, obtained from the rectum by curettage. A bouillon culture is prepared and from this agar tubes are inoculated *as soon as possible.* The agar should be just acid to phenolphthalëin (slightly alkaline to litmus), and is plated at once. Ten plates, variously diluted, are conveniently used. After twenty-four hours in the incubator at 37°–38° C. all colonies are marked on the plates which have developed by that time. The plates are returned to the incubator. After further twenty-four hours tubes of glucose agar and litmus-mannite agar are inoculated from those colonies which have grown in the second twenty-four hours—*i. e.*, those colonies which have not been marked. At the end of another twenty-four hours in the incubator all those tubes are rejected in which fermentation has taken place. From those tubes in which this has not occurred, litmus milk, litmus mannite, and bouillon are inoculated. The Shiga bacillus will at first render the milk slightly acid, but later it becomes alkaline. Litmus mannite remains unchanged with the Shiga strain, while the Harris type (the American form) turns it red. Ultimate identification is made by the agglutination test in various dilutions (1 : 50 to 1 : 100) reading the results after two hours.

LITERATURE.—K. Shiga, Centralbl. f. Bakt., Parasit. u. Infectionskrankh., 1898, vol. xxiv. R. P. Strong and Musgrave, "Preliminary Note regarding the Ætiology of the Dysenteries of Manila," Report of the Surgeon-General of the Army, Washington, 1900, p. 251. S. Flexner, "On the Etiology of Tropical Dysentery," Bull. Johns Hopkins Hosp., 1900, p. 231. Vedder and Duval, "The Etiology of Acute Dysentery in the United States," Jour. Exper. Med., vol. vi. p. 181. Duval and Bassett, Am. Med., 1902, iv. p. 417 (preliminary report).

CHEMISTRY OF THE FECES.

Mucin.—According to Hoppe-Seyler, mucin is a constant constituent of the feces, both under physiological and pathological conditions. Normally, however, it is never possible to recognize its presence either with the naked eye or with the microscope. In order to demonstrate the presence of mucin the feces are digested with water and treated with an equal volume of milk of lime; the mixture is allowed to stand for several hours, when it is filtered and the filtrate tested with acetic acid. In the presence of mucin a cloud develops upon addition of the acid.

Albumin is demonstrated in the feces by treating repeatedly with water slightly acidified with acetic acid. The filtrate is then examined for albumin according to methods given elsewhere (see Urine). Under normal conditions these reactions prove negative. Pathologically, serum-albumin has been observed in cases of typhoid fever and chlorosis.

Peptones (albumoses) are normally absent from the feces. They have been observed in typhoid fever, dysentery, tubercular ulceration, purulent peritonitis with perforation into the gut, atrophic cirrhosis, and carcinoma of the liver. Acholic stools are also usually rich in peptones.

The peptones are demonstrated in the following manner: the feces are digested with water, so as to form a thin mush; they are then boiled, filtered while hot, and the filtrate examined for albumin, so as to be sure that all of this has been removed. The mucin is removed by treating with lead acetate, when the filtrate is examined for peptones as described in the chapter on Urine (which see).

Carbohydrates.—Of the carbohydrates, starch, glucose, and certain gums may be found. In order to demonstrate these the feces are boiled with water, filtered, and evaporated to a small volume. This solution may now be tested with phenylhydrazin or Trommer's reagent for glucose (see Urine), and with a solution of iodo-potassic iodide for starch (see Saliva, page 199). The residue is extracted with alcohol and ether, as described under the heading of fatty acids, and then with water. The filtrate of the aqueous extract is concentrated, boiled with dilute sulphuric acid, and then over-saturated with sodium hydrate. This mixture is treated with cupric sulphate and boiled, in order to test for dextrin and gums.

In normal breast-fed infants sugar is only demonstrable in traces in the stools. Langstein [1] finds that the presence of more than traces of glucose in the stools of milk-fed infants may be regarded as a diagnostic symptom of the localization of a catarrhal process in the duodenum.

Bile-pigment, which is normally absent from the feces, occurs in

[1] L. Langstein, Jahresb. f. Kinderheilk., vol. vi. Heft 3.

large amounts in catarrhal conditions of the small intestine, and may be demonstrated by Gmelin's method, viz., a drop of the filtered liquid, or a particle of highly colored fecal matter, is brought into contact with a drop of fuming nitric acid, when the yellow color will be seen to pass through the various shades of the spectrum, the green shade being the most characteristic. At times, however, it is not possible to obtain a positive reaction in this manner, although bile-pigment is present. In such cases the examination should be conducted under the microscope, and attention directed to bile-stained epithelial cells, leucocytes, particles of mucus, and crystals.

Whenever there is increased intestinal putrefaction the fatty acids, phenol, indol, and skatol will, of course, be found in increased amounts.[1]

Ptomains.—Of ptomains, only two have been isolated from the feces, under pathological conditions, viz., putrescin and cadaverin. They have been found in Asiatic cholera, in cholerina, dysentery, and in connection with cystinuria. In cholera and cystinuria their amount may be quite large. Baumann and v. Udranszky thus obtained 0.5 gramme of the benzoylated compounds from the collected feces of twenty-four hours. In cholera the cadaverin seems to predominate, while in cystinuria more putrescin is found.[1]

To isolate the diamins in question, the feces are digested with alcohol which has been acidified with sulphuric acid. The alcoholic extract is evaporated, the residue dissolved in water. and further benzoylated, as described in the section on Urine.

MECONIUM.

By meconium are meant those masses which are first excreted from the bowel after birth. It is a thick, tenacious, greenish-brown material, which has accumulated during the intra-uterine life of the infant. Microscopically, a few cylindrical epithelial cells, a few fat-droplets, numerous cholesterin-crystals, bilirubin-crystals, and lanugo-hairs are found.

Micro-organisms are absent, but soon after suckling has commenced they appear in abundance. The most important of those which are then constantly present are the Bacillus lactis aërogenes, which predominates in the small intestine, and the Bacillus coli communis, which is found more particularly in the large intestine. Both have already been described (see page 328).[2]

In addition to these, the Proteus vulgaris, Streptococcus coli brevis, Micrococcus ovalis, tetragencoccus, Torula cerevisiæ, Torula rubra, and a few less important micro-organisms have been found.

[1] C. E. Simon, Physiological Chemistry, Lea Bros. & Co., Phila., 1901.
[2] A. E. Austin, "The Chemical Examination of the Feces for Clinical Purposes," Phila. Med. Jour., 1900, p. 551.

Chemically, meconium contains bilirubin in considerable amount (recognizable by Gmelin's reaction), biliary acids, fatty acids, chlorides, sulphates, phosphates of the alkalies and their earths. It does not contain urobilin, glycogen, peptones, lactic acid, tyrosin, or leucin.

An idea may be formed of its composition from the following analysis of Zweifel:[1]

Water	79.8–80.5 per cent.
Solids	19.5–20.2 "
Mineral matter	0.978 "
Cholesterin	0.797 "
Fats	0.772 "

[1] Hellström, Arch. f. Gynäk., 1901, vol. lxiii. Heft 3.

CHAPTER V.

THE NASAL SECRETION.

In the nasal secretion, which normally is small in amount, transparent, colorless, odorless, tenacious, and of a slightly saline taste, pavement-epithelial cells in large numbers, ciliated epithelial cells, as well as some leucocytes and an enormous number of micro-organisms, are found (Fig. 77). Its reaction is alkaline.

Fig. 77.

Epithelial cells and mucous corpuscles found in the nasal secretion.

In acute coryza the amount is diminished at first, but soon a very copious secretion occurs, which contains numerous epithelial cells and micro-organisms. When complicated with an ulcerative condition pus is observed in considerable amount.

Occasionally, as in cases of traumatism, cerebral tumors, etc., cerebrospinal fluid is discharged through the nose, and may be recognized by the fact that it is free from albumin and contains a substance which reduces Fehling's solution.

Of pathogenic organisms, the tubercle bacillus and the bacillus of glanders may occur in ulcerative diseases of the nose, their presence indicating the existence of the corresponding affection. In ozæna a large diplococcus has been described by Löwenberg, which is said to be characteristic of the disease. Oïdium albicans has been observed in rare cases. The Meningococcus intracellularis of Weichselbaum, which is now quite generally regarded as the cause of epidemic cerebrospinal meningitis, has also been demonstrated in the nasal secretion of healthy individuals. This fact helps to explain the origin of those cases of meningitis which develop after injuries to the skull.

Ascarides and other entozoa have also been found. Charcot-Leyden crystals (see page 364) have been observed in the nasal secretion in cases of bronchial asthma and in connection with nasal polypi. Their presence is usually accompanied by the simultaneous occurrence of eosinophilic leucocytes.

LITERATURE.—Reimann, Baumgarten's Jahresber., 1888, vol. iii. p. 417. Löwenberg, Deutsch. med. Woch., 1885, vol. xi. p. 6, and 1886, vol. xii. p. 446. Tost, Ibid., p. 161. Gerber u. Podack, Deutsch. Arch. f. klin. Med., 1895, vol. liv. p. 262. Leyden, Deutsch. med. Woch., 1891, vol. xvii. p. 1085. Sticker, Zeit. f. klin. Med., 1888, vol. xiv. p. 81. Nothnagel, Wien. med. Blätter, 1888, Nos. 6, 7, 8.

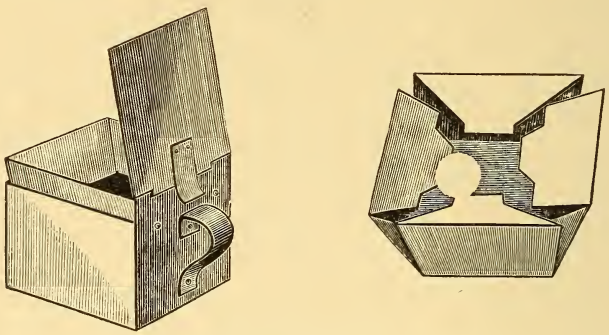
CHAPTER VI.

THE SPUTUM.

GENERAL TECHNIQUE.

THE sputum should be collected in receptacles so constructed as to permit of their complete and easy disinfection. The paper spit-cups (Fig. 78) which have been introduced within late years are admirably adapted to this purpose, as they may be destroyed immediately after use.

FIG. 78.



Sanitary spit-cups.

When working with sputa which are known or suspected to be of tubercular origin, the greatest care should be exercised to keep the expectoration from drying and becoming disseminated in the air. Negligence in this respect may result in the most serious consequences.

The macroscopical examination of sputa is most conveniently carried out by placing small portions of the material upon a plate of ordinary window-glass, of suitable size, which has been painted black upon its lower surface, and covering the same with a second, smaller plate. If it is desired to examine individual constituents which have been discovered in this manner, the upper plate is slid off until the particle in question is uncovered, when it may be removed to a microscopical slide and examined under a higher power.

It is also very convenient to have a portion of the laboratory table painted black, when unstained plates of glass may be utilized. If these measure about 15 by 15 cm. and 10 by 10 cm., respectively, fairly large quantities of sputum may be examined *in situ* with a low power.

GENERAL CHARACTERISTICS OF SPUTA.

Amount.—The amount of sputum expectorated in the twenty-four hours varies within wide limits, depending largely upon the nature of the disease. Thus, only a few cubic centimeters may be eliminated, or the amount may reach 600 to 1000 c.c., and even more. Very large quantities are expectorated in cases of pulmonary hemorrhage and œdema of the lungs, sometimes following thoracentesis, also following perforation of accumulations of pus from the thoracic or abdominal cavities into the respiratory passages; furthermore, in cases in which large vomicæ of tubercular or gangrenous origin exist, and finally in cases of abscess of the lung, bronchiectasis, and even in simple bronchial blennorrhœa. In incipient phthisis, acute bronchitis, and in the first and second stages of pneumonia, on the other hand, the amount is usually small.

In private practice, as well as in hospital work, an idea should always be formed of the amount expectorated in the twenty-four hours, especially in cases in which this is abundant. It is apparent that a copious and long-continued expectoration cannot continue without exerting very detrimental effects upon the patient's general nutrition; in cases of pulmonary phthisis, for example, Renk has shown that 3.8 per cent. of all nitrogen eliminated in such cases is removed in this manner. Lenz in his recent experiments found even 5 per cent.

Consistence.—The consistence of the sputum corresponds, in a general way at least, to its amount, and may vary from a liquid to a highly tenacious state. The cause of the tenacity of the sputum is but imperfectly understood. The mucin present does not appear to be the most important factor, as it has been observed to occur in diminished amount in pneumonic sputa, which are noted for their high degree of tenacity. Kossel[1] has suggested that the phenomenon may be due to the presence of nucleins or nuclein derivatives, while others again refer it to the presence of abnormal albuminous bodies of unknown character. However this may be, sputa are not infrequently seen where it is possible to invert the cup without losing a drop of its contents. This is observed especially in cases of acute croupous pneumonia up to the time of the crisis, providing that a catarrh of the bronchi does not exist at the same time. It is noted, furthermore, immediately after an attack of acute bronchial asthma, and also in the initial stage of acute bronchitis. In cases of œdema of the lungs, on the other hand, the sputa are liquid and present the general characteristics of blood-serum, being covered, like all albuminous liquids when brought into contact with the air, by a frothy surface-layer. The sputa observed in cases of acute pulmonary gangrene, pulmonary abscess, putrid bronchitis, and

[1] Kossel, Zeit. f. klin. Med., 1888, vol. xiii. p. 152.

following perforation into the lungs of an empyema or an accumulation of pus situated beneath the diaphragm, are fluid and consist of pure pus.

Color.—The color of the sputa may vary greatly. They may be perfectly clear and transparent, gray, yellow, green, red, brown, and even black. Purely mucoid expectoration is almost transparent and colorless, as is also the sputum of pulmonary œdema when not mixed with blood or pus.

The larger the number of leucocytes the more opaque does the sputum become, assuming at first a white, then a yellow, and finally a greenish color, the two latter colors being usually indicative of the presence of pus. Green sputa, however, may also be observed when bile-pigment has become admixed with the sputa, as in cases of perforation of a liver-abscess into the lung. Green sputa may also be observed in cases of jaundice, and especially in pneumonia when accompanied by icterus. In cases of amœbic liver-abscess with perforation into the lung the sputa present a color resembling anchovy sauce, which is very characteristic. In one case I recognized the nature of the disease by simple inspection of the sputa.[1]

The inhalation of particles of carbon gives the sputum a grayish or even a black color; the same or an ochre-yellow or red color is observed in cases of siderosis.

A red color is usually indicative of the presence of *blood*, the intensity of the shade depending upon the character of the disease. It is seen especially after the formation of cavities, in caseous pneumonia, in incipient phthisis, heart-disease, etc. In general, it may be said that a clear, bright-red color indicates an arterial, a dark-red or bluish-red a venous origin of the hemorrhage. The exact shade will depend upon the length of time that the blood, no matter what its origin may be, has remained in the lungs. In pulmonary gangrene a dirty brownish-red color is observed, owing to the presence of methæmoglobin, and, to some extent also, of hæmatin. Quite characteristic is a chocolate color, which is observed when a croupous pneumonia terminates in necrosis and gangrene. Equally characteristic is the rusty and prune-colored expectoration seen in cases of pneumonia. Occasionally a breadcrust-brown color is observed in cases of gangrene and abscess of the lung, which is quite characteristic, the color being due to the presence of hæmatoidin or bilirubin.

Rust-colored punctate or striped sputa, moreover, are said to be diagnostic of brown induration of the lung.

Odor.—Most sputa are odorless. Under certain conditions, however, there may be a very marked odor. In cases of pulmonary gangrene or putrid bronchitis the odor is of a kind never to be forgotten, the stench, indeed, being frightful. A somewhat similar,

[1] See Johns Hopkins Hosp. Bull., November, 1890.

slightly sweetish odor is observed in certain cases in which putrefactive organisms have entered the lungs, and there exert their action upon the accumulated sputa, in the absence of gangrene, as in cases of bronchiectasis, perforating empyema, and where ulcerative processes are taking place in the lungs, whether these be of tubercular origin or not. An odor like that of old cheese is occasionally observed in cases of perforating empyema; under such conditions tyrosin is usually found. This body, however, has nothing to do with the odor of the sputa; both factors are merely indicative of certain putrefactive changes going on in the lungs. According to Leyden, the occurrence of tyrosin in sputa is usually indicative of the perforation of an old accumulation of pus into the lungs.

Specific Gravity.—The specific gravity of sputa varies within wide limits; mucous sputa have a specific gravity of 1.004 to 1.008, purulent sputa one of 1.015 to 1.026, and serous sputa one of 1.037 or more.

Configuration of Sputa.—As a general rule, the following forms of sputa, which may be termed pure sputa, present a homogeneous appearance:

Mucoid sputa, Purulent sputa, Serous sputa, Sanguineous sputa,	Homogeneous sputa,

with one exception, perhaps—the typically rusty sputa of croupous pneumonia; while mixtures of any two or three of these may be classed as heterogeneous sputa:

Mucopurulent sputa, Mucoserous sputa, Serosanguineous sputa, Sanguino-mucopurulent sputa,	Heterogeneous sputa.

The so-called *sputum crudum* of the first stage of acute bronchitis may be regarded as an example of a purely mucoid sputum. A purely purulent sputum is usually indicative of the perforation of an empyema or any other accumulation of pus into the lungs or bronchi, of pulmonary abscess, or of bronchial blennorrhœa. A purely serous sputum is found in cases of pulmonary œdema, and a purely hemorrhagic sputum in cases of severe pulmonary hemorrhage.

Of the heterogeneous sputa, the most important are the so-called *nummular sputa* of the second and third stages of phthisis. These are characterized by the fact that when thrown or expectorated into water they sink to the bottom, and there form coin-like disks, from which property they have received their name. Such sputa are mucopurulent in character, and contain a focus of almost pure pus imbedded in a more or less homogeneous mass of mucus. Quite different from these are the so-called *sputa globosa* of the ancients, which consist of fairly dense, roundish, grayish-white masses; they

are secreted in old cavities which have become lined with a granulation-membrane.

Very important is the presence of small, *cheesy particles*, which are occasionally found at the bottom of the spit-cup. They vary in size from that of a millet-seed to that of a pea, and are observed especially in the second and third stages of phthisis. Usually they contain tubercle bacilli in large numbers, and frequently also elastic tissue. Not to be confounded with these, are certain small, caseous masses which are at times expectorated by perfectly normal individuals, and also by patients suffering from acute tonsillitis, ozæna, etc., and which probably come from the tonsils or mucous cysts. Formerly they were regarded as tubercles, and in hypochondriac individuals their expectoration may cause a great deal of anxiety. They are quite readily distinguished from the true caseous masses expectorated by phthisical individuals by the following characteristics: as a rule, they are expectorated unaccompanied by pus or even by mucus; rubbed between the fingers they emit an extremely offensive odor, which is referable to the presence of fatty acids; an examination for tubercle bacilli, moreover, will prove entirely negative. Quite characteristic, furthermore, is the peculiar, finely flocculent, granular appearance of the sputa seen after perforation of an empyema into the lungs through a small aperture, which is not followed by pneumothorax.

Occasionally, as in putrid bronchitis, and gangrene of the lungs, and also in chronic bronchitis, ultimately leading to the formation of bronchiectatic cavities, an exquisite *sedimentation* is observed. Such sputa when collected in a conical glass present three distinct zones: the one at the bottom contains the cellular elements of the sputum, the second the pus-serum; and the third or superficial layer consists of mucus and contains many air-bubbles.

MACROSCOPICAL CONSTITUENTS OF SPUTA.

Elastic Tissue.—Of macroscopical constituents which may be observed in sputa, there may be mentioned, first of all, the occurrence of threads of elastic tissue and pulmonary parenchyma, which are seen in cases of phthisis, pulmonary abscess, and gangrene. As their ultimate recognition, however, largely depends upon a microscopical examination, this subject will be considered later on.

Fibrinous Casts.—Fibrinous casts are observed especially in cases of croupous pneumonia (Fig. 79), immediately before or after resolution has taken place. They are seen also in cases of so-called fibrinous bronchitis (Fig. 80), and in diphtheria when the membrane has extended into the finest ramifications of the bronchi. These casts may vary in size from 15 cm. in length by several millimeters in thickness to fragments which measure only from 0.5 to 3

cm. in length. The fibrinous casts observed in cases of pneumonia, usually from the third to the seventh day, are of the latter size or even smaller, being derived from the ultimate twigs of the finest bronchioles. Those found in the rather rare disease, fibrinous bronchitis, stand between these two in size, being casts of the smaller and medium-sized bronchi. Attention is usually attracted to the presence of such casts by their white color; often, however, they are yellowish brown or reddish yellow, owing to the presence of blood-coloring matter which has become deposited upon the casts; at other times they are enveloped in mucus, when their recognition may become

FIG. 79.

Fibrinous coagulum from a case of croupous pneumonia. (BIZZOZERO.)

quite difficult. Such casts are fairly firm; they branch dichotomously, usually 6 to 10 times. The larger branches contain a lumen, while the smallest twigs are solid. Microscopically they may be shown to consist of a large number of fibres, which are arranged longitudinally or in a net-like manner, and contain blood-corpuscles and epithelial cells in their meshes. When treated with Weigert's fibrin-stain, they are sometimes beautifully resolved; at other times the fibrin reaction is not nearly so marked as one would expect. The individual casts consist of a variable number of laminæ arranged concentrically, those contained in the centre being much folded and involuted. Most of the branches are cylindrical; some

of the larger ones are flat. Charcot-Leyden crystals have at times been observed in these formations.

Fig. 80.

Expectorated cast, from a case of fibrinous bronchitis. Three-fourths natural size. Drawn from fresh specimen. (After Bettmann.)

Whenever it is desired to examine sputa for casts, it is best to pick out particles that look promising, upon a dark or light surface, and then to shake them out in water. For such purposes Krönig's sputum-plate can be recommended.

Literature.—M. Bettmann, Am. Jour. Med. Sci., 1902, vol. cxxiii. p. 304 (a full review of all cases in the literature up to 1902 is here given).

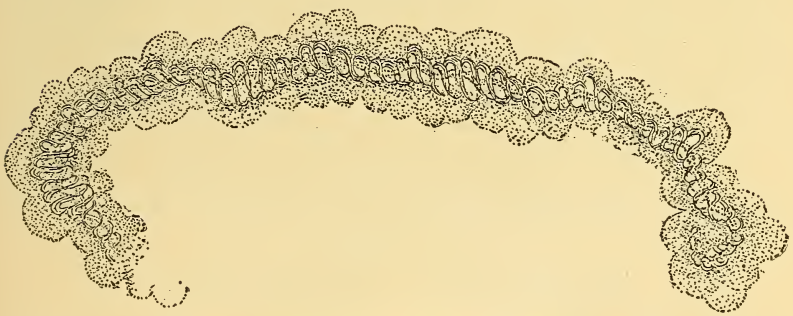
Curschmann's Spirals.[1]—Quite distinct from the formations just described are the so-called spirals of Curschmann, which are observed especially in cases of true bronchial asthma, but occur also in chronic bronchitis, and even in croupous pneumonia. Upon

[1] Leyden, Virchow's Archiv, 1872, vol. liv. p. 328. Curschmann, Deutsch Arch. f. klin. Med., 1883, vol. xxxii. p. 1, and vol. xxxvi. p. 578. v. Jaksch, Centralbl. f. klin. Med., 1883, vol. iv. p. 497.

careful examination they will be seen to consist of thick, yellowish-white masses, which exhibit a spirally twisted appearance, and are characterized, moreover, by their more solid consistence and light color. On microscopical examination they are seen to be composed of a spirally twisted network of extremely delicate fibrils, containing epithelial cells and numerous leucocytes ; the latter are almost all of the eosinophilic variety.[1] Usually, but not invariably, Charcot-Leyden crystals also are seen.[2] The spirally twisted mass is found to be wound around a central, very light and clear thread, which usually has a zigzag course (Fig. 81).

Other formations, probably mere varieties of those just described, have also been observed, in which the central thread is absent or in which the spiral arrangement is deficient. The spiral form, however, with the central thread, must be considered as the most characteristic. Their length and breadth may vary a great deal, but rarely exceed 1 to 1.5 cm. Their occurrence seems always to indicate a desquamative catarrh of the bronchi and alveoli, but practically nothing is known concerning their formation. If in a given case the diagnosis rests between true bronchial and what may be termed reflex asthma, the presence of these formations points to the existence of the former disease. Chemically, the spirally wound

FIG. 81.



A Curschmann spiral from a case of true bronchial asthma.

mass seems to consist of a mucinous substance, while the central thread is possibly of fibrinous origin.

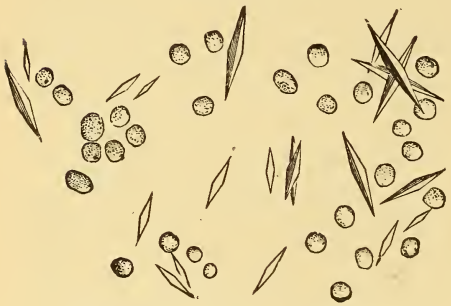
Charcot-Leyden crystals (Fig. 82), which are usually absent at the beginning of an attack of asthma, at which time only the spirals are observed, may be seen to develop from the spirals when these are kept for several days. They will be considered later in studying the chemistry of the sputum.

Echinococcus Membranes.—Echinococcus membranes come

[1] Schmidt, Zeit. f. klin. Med., 1892, vol. xx. p. 92. v. Noorden, Ibid., p. 98.
[2] Leyden, loc. cit.

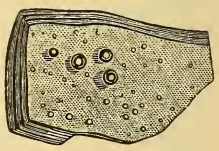
from a perforating cyst of the liver, kidney, or lung. They constitute rather thick, and at the same time tough, pieces of membrane (Fig. 83); occasionally entire sacs are seen, of the color of white porcelain, in sections of which it is possible to make out a fibrillated structure. The disease is rare in this country.

FIG. 82.



Charcot-Leyden crystals. (SCHEUBE.)

FIG. 83.



Wall of a hydatid cast, showing the laminated structure; not magnified. (DAVAINE.)

Concretions.—Still rarer is the expectoration of concretions which have formed in dilated portions of the bronchi or in tubercular cavities, or of calcified bronchial glands that have found their way into the lungs. Curious examples of the occurrence of such concretions have been reported. Andral thus cites a case of phthisis in which within eight months as many as 200 stones were expectorated, and Portal mentions a case in which 500 were thus expelled.[1]

Foreign Bodies.—Foreign bodies which have accidentally entered the air-passages and have remained there for a long time may also be found in the sputum. Heyfelder mentions a case in which a man coughed up a wooden cigar-holder with pus and blood after eleven and a half years.

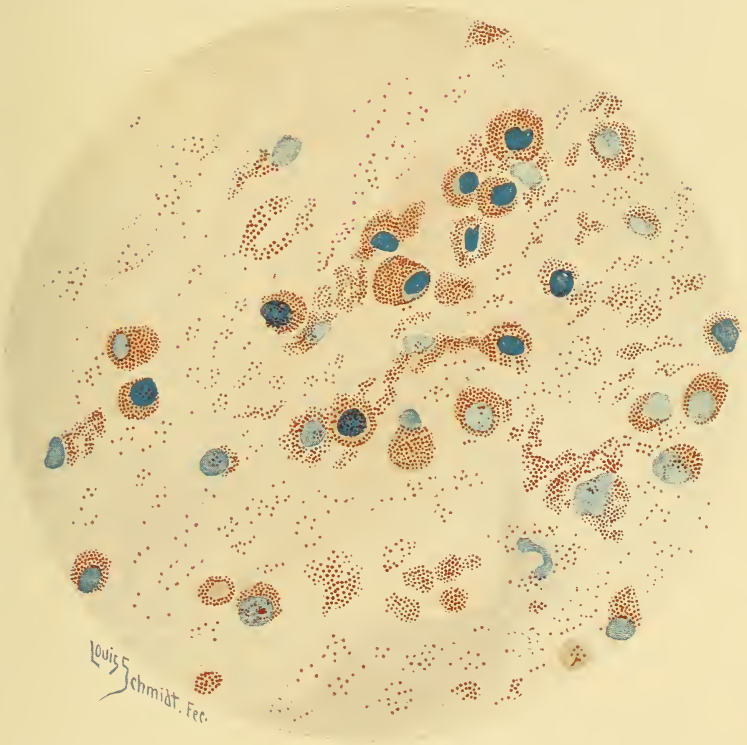
MICROSCOPICAL EXAMINATION.

Under this heading it is necessary to consider leucocytes, red blood-corpuscles, epithelial cells, elastic fibres, corpora amylacea, parasites, and crystals.

Leucocytes.—Leucocytes, usually polynuclear in character, are found in every sputum in considerable numbers, imbedded in a homogeneous, more or less tenacious material. At times they appear very granular, containing fat-droplets, or granules of pigment, such as carbon or hematoidin. Their number varies considerably, being naturally greatest in cases of perforating abscess, empyema, putrid bronchitis, etc.

[1] L. W. Atlee, "Bronchial Concretions," Am. Jour. Med. Sci., 1901, vol. cxxii. p. 49. Fiessinger, "Calcule pulmonaire," Jour. de Méd., 1902, No. 29.

PLATE XVI.



Sputum from a case of Bronchial Asthma, showing large numbers of Eosinophilic Leucocytes and Free Granules.

It will be noted that the leucocytes are all mononuclear. (Eye-piece 1, objective 1-8, Bausch and Lomb.)

While the leucocytes which usually are found in the sputum are of the neutrophilic variety, eosinophiles may also be observed, and especially in asthmatic sputa, in which they often predominate. Free eosinophilic granules are then also seen, and I have repeatedly observed specimens in which the spirals (see above) were literally covered with these granules (Plate XVI.). The presence of eosinophilic leucocytes is, however, not characteristic of the sputa of bronchial asthma, as they may be met with in other diseases as well. Teichmüller has pointed out that they are present in a large percentage of tubercular cases, and may be found months before tubercle bacilli can be demonstrated. He regards their occurrence as evidence of a defensive struggle on the part of the body, which is most evident in fairly strong individuals. In recovery a gradual increase in their number is always noticeable, and a diminution, Teichmüller thinks, is indicative of a relapse, or, if the diminution occurs rapidly, of florid consumption. These statements, however, lack confirmation and are probably too dogmatic. Ott, Fuchs, Bettmann, Turban, and Cohn, in fact, deny the prognostic significance of the eosinophilic cells in cases of phthisis; and Cohn states as the result of an examination of 100 cases, many of which were comparatively early, that the occurrence of eosinophilic leucocytes is fairly uncommon in tubercular sputa.[1]

Stadelmann also states that he has been unable to verify Teichmüller's observations. On the other hand, he has been able to confirm the observation which has been repeatedly made, that large numbers of eosinophilic cells appear in the sputum following hæmoptysis. Teichmüller has also described an "eosinophilic" bronchitis, which is said to differ from other forms of the disease in the abundance of eosinophilic cells which are encountered. The sputum in such cases is described as transparent, mucoid, and loose, with yellow purulent admixtures. It is said to be markedly different from the tough, thick sputa of bronchial asthma. Typical spirals are absent, but rudimentary forms may be encountered. Charcot-Leyden crystals are present.[2]

Grünwald[3] states that in the sputa of the most diverse diseases cells are met with which contain a hypoeosinophilic granulation, and that the granules in question may also occur outside of the cells in the absence of evidence of special cell-destruction. These granules, in contradistinction to the true eosinophilic cells, lose their color on treating with an acid, and readily take up the blue stain on

[1] Discussion on tuberculosis, Deutsch. med. Woch., 1901, V. B. p. 210.

[2] Teichmüller, "Die eosinophile Bronchitis," Deutsch. Arch. f. klin. Med., vol. lxiii. p. 444. See, also, K. Schönbrod, Ueber den gegenwärtigen Stand der Beurtheilung der eosinophilen Zellen im Blute und im Sputum, Inaug. Diss., Erlangen, 1895. A. Hein, Ueber das Vorkommen eosinophiler Zellen im Sputum, Inaug. Diss., Erlangen, 1894.

[3] L. Grünwald, "Studien über d. Zellen im Auswurf," etc., Virchow's Archiv, 1899, vol. clviii. p. 297.

subsequent staining with methylene-blue. Grünwald states, however, that a sharp line of distinction does not exist between the two varieties of granules, and that intermediary conditions exist, as also transitions between oxyphilic and basophilic granules in the nature of an amphophilic granulation.

To demonstrate eosinophilic leucocytes in the sputum, smears are made as usual, slightly fixed by drawing through the flame of a burner, and stained for two minutes in a 0.5 per cent. dilute alcoholic solution of eosin. The preparations are then immersed in 50 per cent. alcohol to the point of decolorization, when they are counterstained with methylene-blue, briefly washed with water, and dried. The eosinophilic granules and the red cells in part hold the eosin dye.

Basophilic leucocytes have also been observed in the sputa.

Red Blood-corpuscles.—The presence of red blood-corpuscles in small numbers does not, by any means, indicate serious pulmonary or cardiac disease, as they may be found in almost any sputum, and especially in that of individuals who smoke much or live in a smoky atmosphere; they are, without doubt, derived from the catarrhally inflamed bronchial or tracheal mucosa. Whenever they occur in large numbers, however, their presence becomes important. They may be observed in acute bronchitis, pneumonia, œdema of the lungs, bronchiectasis, abscess, gangrene—in fact, in all pulmonary diseases. Their occurrence is most important in phthisis, and is, in fact, one of the most constant symptoms of the disease.

The form of the red corpuscles will depend upon the length of time they have remained in the lungs, and all gradations from the typical red corpuscle to its shadow, or even fragments, may thus be observed. In pneumonia the microscopical examination may at times be disappointing, the appearance of the sputum suggesting that red corpuscles in large numbers are present, while, as a matter of fact, they are almost all destroyed, the color being due to altered pigment. It may even be necessary at times to depend upon chemical methods to clear up any doubt as to the source of the color of the sputum. It should always be remembered that the presence of blood-pigment is not always indicated by a red color, but that it may also assume a golden-yellow or even a greenish tinge, owing to certain chemical changes which have taken place. The golden-yellow and the grass-green sputa observed in cases of pneumonia during convalescence belong to this class.

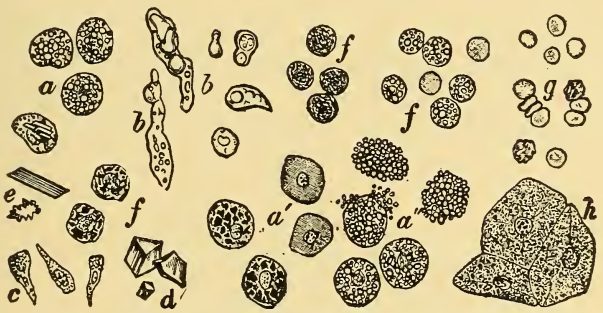
To demonstrate the presence of traces of blood in the sputum, Donogany's method, or that of Müller and Weber, may be conveniently employed. With the former method the sputum is first boiled with a 20 per cent. solution of sodium hydrate (see page 262).

Epithelial Cells.—Epithelial cells are found in practically every sputum. Cylindrical epithelial cells, providing they do not come from the nose, indicate in a general way an inflammatory condition

of the lower larynx, trachea, or bronchi. They are not of much importance, however, as their form is usually so much altered that it is often difficult to recognize them ; they may thus become polyhedral, cuboidal, or even round, and can then hardly be distinguished from leucocytes. Actively moving cilia may be found only in perfectly fresh sputa, immediately after being expectorated. If ciliated epithelial cells can be definitely recognized in a sputum, it may be inferred that we are dealing with a pathological condition of an acute nature, providing, of course, they did not come from the nose.

Formerly much importance was attached to the so-called *alveolar epithelial cells* (Fig. 84) as an aid in diagnosis. Buhl thus imagined these, particularly when undergoing fatty or myelin degeneration, to be absolutely pathognomonic of pulmonary disease, and especially of that form of pneumonia which has been termed essential idiopathic desquamative pneumonia. Bizzozero, however, as well as

FIG. 84.



Epithelium, leucocytes, and crystals of the sputum. (Eye-piece III., objective 8 A, Reichert.) *a*, *a'*, *a''*, alveolar epithelium ; *b*, myelin forms ; *c*, ciliated epithelium ; *d*, crystals of calcium carbonate ; *e*, hæmatoidin crystals and masses ; *f*, *f*, *f*, white blood-corpuscles ; *g*, red blood-corpuscles ; *h*, squamous epithelium. (v. JAKSCH.)

others, has shown that these cells not only occur in almost every known pulmonary disease, but that they are present also in the so-called "normal" expectoration which at times is obtained upon making a very forcible expiration.

Bizzozero [1] describes these cells as round, oval, or polygonal bodies, varying in size from 20 μ to 50 μ. They may contain one, two, or three oval nuclei, which are rather small and provided with nucleoli. Usually the latter are hidden beneath numerous granules. Some of these granules are albuminous, but most of them are either pigment-granules, fatty granules, or myelin granules. The *myelin granules* were first discovered by Virchow [2] in 1854, and termed myelin granules on account of their resemblance to mashed nerve-matter. They are distinguished from the other forms by their clear, pale, color-

[1] Bizzozero, Microscopie clinique, 2d ed. Française, Paris, 1885.
[2] Virchow, Virchow's Archiv, 1854, vol. vi. p. 562.

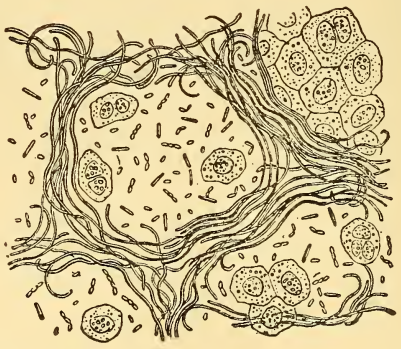
less appearance, and the fact that at times fine concentric striations can be detected. These forms may be round, but more often they are irregular. At times fatty, myelin, and pigment-granules may be seen in one and the same cell. Possibly they are derived from the pulmonary alveoli, but this is still an open question. Chemically, the myelin droplets have been shown to contain a considerable amount of protagon, besides traces of lecithin and cholesterin.[1]

The sputa of chronic bronchitis referable to heart disease are characterized by the presence of so-called *heart disease cells*. These are alveolar epithelial cells containing numerous hæmatoidin granules (Plate XVII., Fig. 3). They appear to be most numerous in cases of mitral disease, but may also occur in congestive affections of the broncho-pulmonary apparatus, even with the heart intact.[2]

Liver-cells may at times be observed in the sputa in cases of liver-abscess, and are easily recognized by their characteristic form.

Elastic Tissue.—Much more important from a clinical standpoint are the elastic fibres and shreds of elastic tissue which may be found in sputa. They vary much in length and breadth, and are provided with a double, undulating contour; they are usually curled at their ends. Very often they exhibit an alveolar arrangement (Fig. 85), which at once determines their origin.

Fig. 85.



Elastic fibres in the sputum. (Eye-piece III., objective 8 A, Reichert.) (v. Jaksch.)

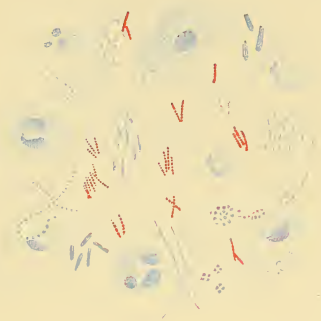
Whenever present, elastic tissue is an absolute indication that a destructive process is going on in the lungs. It is found in cases of abscess of the lungs, bronchiectasis, occasionally in pneumonia, pulmonary gangrene and infarct, and, most important of all, in phthisis, in which it is said to be present in 90 per cent. of all cases. This

[1] A. Schmidt, "Ueber Herkunft u. chem. Natur d. Myeliuformen d. Sputums," Berlin. klin. Woch., 1898, p. 73. See, also, Zoja, Maly's Jahresberichte. vol. xxiv. p. 694.

[2] R. C. Regolo, Gaz. d. Ospedali, Milano, vol. xxii. No. 135.

PLATE XVII.

FIG. 1.



Tuberculous Sputum Stained by Gabbett's Method. The Turbercle Bacilli are seen as Red Rods, all else is Stained Blue. (Abbott.)

FIG. 2.

The Diplococcus Pneumoniæ, Stained with Methylene Blue and Fuchsin as a Counterstain. Taken from the Sputum of a Case of Acute Croupous Pneumonia.

FIG. 3.

Heart-Disease Cells, showing Alveolar Epithelial Cells, Loaded Down with Granules of Hæmatin.

percentage, which was obtained by Dettweiler and Setzer in 1878, is unquestionably too high in comparison to what is seen to-day when the diagnosis of tuberculosis is after all made much earlier. In gangrene of the lung elastic tissue is usually not found; this is probably owing to its destruction by a ferment, as suggested by Traube.

In every case it is necessary to determine whether the elastic tissue may not be owing to the presence of animal food in the sputum, and it may hence be stated as a rule that it can only be regarded as absolutely characteristic when showing the alveolar arrangement.

In order to demonstrate the presence of elastic tissue in the sputum the following method, in use at the Johns Hopkins Hospital, is very convenient: a small amount of the thick, purulent portion of the sputum is pressed into a thin layer between two pieces of plain window-glass, 15 by 15 cm. and 10 by 10 cm. The particles of elastic tissue appear on a black background as grayish-yellow spots, and can be examined *in situ* under a low power. Or, the upper piece of glass is slid off till the piece of tissue is uncovered, when it is picked out and examined on a slide, first with a low and then with a higher power. At first there will be some difficulty in distinguishing with the naked eye between elastic fibres and particles of bread, or milk globules, or collections of epithelium and débris, but with practice such mistakes are rarely made, and the microscope always reveals the difference.

If only very little elastic tissue is present, it is necessary to examine large quantities of sputum with a moderately low power, and best after the addition of a solution of sodium hydrate. The sputum is boiled with a 10 per cent. solution of the reagent, an equal volume being added; the boiling is continued until a homogeneous solution has been obtained; after dilution with four times its volume of water it is allowed to settle for twenty-four hours. The centrifugal machine will here be found of great assistance. The elastic tissue fibres are found in the sediment.

To stain elastic tissue, Michaelis suggests the following method: suspected bits of sputum are spread upon a slide in a thin layer, dried, and then placed for one-half hour in a jar containing Weigert's solution. The specimen is then washed with water, decolorized in acid alcohol (containing 3 per cent. of hydrochloric acid), dried, covered with a thin layer of oil of cedar, and examined without a cover-glass with a low power; the elastic fibres are stained a dark violet.

Weigert's Elastic Tissue Stain.—This is prepared as follows: 200 c.c. of an aqueous solution of fuchsin and resorcin, containing 1 and 2 per cent. of the ingredients, respectively, are boiled in a porcelain dish. When the boiling-point is reached 25 c.c. of liquor ferri sesquichloridi (Ph. G. III.) are added. While stirring the

solution is boiled for from two to five minutes longer. It is then allowed to cool; the precipitate is collected on a filter, dried, and boiled in 200 c.c. of 94 per cent. alcohol while stirring. On cooling, alcohol is added to the 200 c.c. mark, when the solution is treated with 4 c.c. of hydrochloric acid, and is ready for use.

May[1] recommends the following method of demonstrating the presence of elastic tissue in sputum: The material in question is heated on a boiling water-bath with an equal volume of a 10 per cent. solution of sodium hydrate until it has all apparently dissolved. The mixture is then centrifugalized and the supernatant fluid decanted. The sediment is treated with about 2 c.c. of an orceïn solution prepared according to the formula of Unna-Tänzer, viz., orceïn, 1 gramme; absolute alcohol, 80 c.c.; distilled water, 40 c.c.; concentrated hydrochloric acid, 40 drops. On adding the stain, owing to the remaining alkali, the color turns violet; a few drops (3–5) of hydrochloric acid are added until the original color of the stain returns. The tube is then placed for from two to five minutes in boiling water, after which acid alcohol (concentrated hydrochloric acid, 5 c.c.; 95 per cent. alcohol, 1000 c.c.; distilled water, 250 c.c.) is added to decolorize. The mixture is again centrifugalized and the sediment washed once or twice more with the acid alcohol by centrifugation and decantation. The sediment is then examined directly, when the elastic tissue fibres may be recognized by their more or less intense brownish-violet color.

Animal Parasites.

Tænia Echinococcus.—Portions of echinococcus cysts, viz., pieces of membrane (Fig. 84) and hooklets (Fig. 86), are occasionally seen when the parasite has lodged in the lungs or in the neighboring organs. The disease is not common in this country. Lyon[2] has collected a total of 241 cases in the United States and Canada up to July 1, 1901. 91 per cent. occurred in foreigners. In Canada a large proportion is referable to the Icelandic immigrants in Manitoba.

The adult parasite (Fig. 87) (v. Siebold) is found in the intestinal canal of the dog, the dingo, the jackal, the wolf, etc. The larval form, *Echinococcus polymorphus*, develops in cattle, sheep, and swine, and is also found in man. The parasite, in fact, is the most dangerous animal parasite which is encountered in the human being. If the eggs of the parasite are introduced into the digestive tract of man, the embryos may make their way into the lungs, liver, or other organs, and there give rise to the formation of cysts, which are often of enormous size. The body of the adult animal is from

[1] R. May, Deutsch. Arch. f. klin. Med., 1900, vol. lxviii. p. 427.
[2] I. P. Lyon, N. Y. State Jour. Med., Oct., 1902.

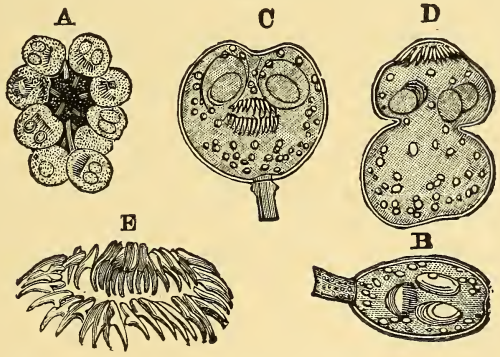
4 to 5 mm. long, with only 3 or 4 segments, the largest of which may measure 0.6 mm. in length by 2 mm. in breadth. On the head there are from 28 to 50 hooklets (see Fig. 87).[1]

Fig. 86.

Hooklets from Tænia echinococcus. × 350.

Trichomonades have at times been observed in cases of gangrene of the lung, and in the pus removed post mortem from lung-cavities. They are identical with the Trichomonas vaginalis of Donné.

Fig. 87.



Human echinococcus. (From Finlayson, after Davaine.) *A*, a group of echinococci, still adhering to the germinal membrane by their pedicles. × 40. *B*, an echinococcus with head invaginated in the body. × 107. *C*, the same compressed, showing suckers and hooks of the retracted head. *D*, echinococcus with head protruded. *E*, crown of hooks, showing the two circles. × 350.

Amœba Coli.—The presence of this parasite is most important, as the diagnosis of hepatic abscess with perforation into the lung may be made in every instance in which the organism is encountered in the sputa (see Feces).[2]

Distoma Pulmonale.—A form of pulmonary disease closely sim-

[1] Hydatid disease in man: Neisser, Die Echinococcen-Krankheit, 1877, Berlin. Davaine, Traité des Entozoaires et des Maladies vermineuses, Paris, 1877, 2d ed.

[2] C. E. Simon, Johns Hopkins Hosp. Bull., Nov., 1890.

ulating phthisis is very common in Japan, and has been shown to be referable to the presence of a parasite in the lungs, Distoma pulmonale Bälz (*syn.*, Distoma Westermanni (Kerbert), Distoma Ringeri (Cobbold)). The parasite is 8 to 10 mm. long, 4 to 6 mm. wide, rounded very markedly in front, less so posteriorly. The color during life is a reddish-brown. The two sucking disks are nearly equal in size. The ova are brown, with a thin shell and lidded. They measure from 80 to 100 μ in length and 40 to 60 μ in breadth. The worm and its ova are found in the sputum. If the sputum is shaken in water and the water renewed from time to time, in the course of a month or six weeks (according to the temperature) a ciliated embryo is developed in each ovum. When the ovum is mature, on placing it on a slide and exercising slight pressure on the cover-glass, the operculum will be forced back, and the embryo will emerge and at once begin to swim and gyrate in the water (Manson). Outside of Japan the parasite has been found in Corea and Formosa. In the United States it has been found in the cat and in the dog; in the human being one case at least, occurring in a Japanese student, has been reported. Many Charcot-Leyden crystals are found in the sputum at the same time.

Literature.—C. D. Stiles, "Distoma Westermanni," Johns Hopkins Hosp. Bull., 1894, p. 57. Brown, Die thierischen Parasiten, etc., Stuber, Würzburg, 1895.

Distoma Hæmatobium.—Manson found the ova of a species of Distoma hæmatobium in the bloody expectoration of a Chinaman who had lived for some time on the island of Formosa.

Vegetable Parasites.

Pathogenic Organisms.—The Tubercle Bacillus.—The most important vegetable parasite met with in the sputa is the *bacillus of tuberculosis*. The history of the discovery of this organism, and the theories which were held before its pathogenic importance was established, cannot be considered here. Suffice it to say that the study of bacteriology has given no other discovery of equal importance from a clinical point of view. How primitive and wholly inadequate were the means formerly employed in making the diagnosis of this, the most formidable disease of modern times! The presence or absence of elastic tissue in the sputa was practically all that physicians had to guide them beyond the history of the patient and the results of a physical examination. The demonstration of elastic tissue, however, as has been pointed out, merely indicates the existence of a destructive process in the lungs. Under such conditions it was of necessity impossible to diagnose tubercular disease in its incipiency. It is true that cases are occasionally observed in which tubercle bacilli are never present in the sputa, and are only discovered post mortem. Such cases, however, are extremely rare,

and do not in the least detract from the importance which attaches to careful and repeated examinations of the sputa in all doubtful cases.

From a macroscopical examination it is impossible to decide whether or not a particular sputum is of tubercular origin. At times a sputum may have a suspicious appearance, but it is never possible to speak with certainty from simple inspection, as a mucoid sputum may contain tubercle bacilli in large numbers, while a muco-purulent sputum may be entirely free from them, and *vice versa*. Reliance should, hence, only be placed upon a careful microscopical examination. When found, their presence is, of course, pathognomonic. A negative result, however, does *not* exclude the existence of tubercular disease. The possibility that they may be altogether absent from the *sputum* has been mentioned. In some instances they may be present at times and absent at others. In all cases in which the existence of phthisis is suspected, it is imperative to make use of every device which may aid in its detection. In this connection, I wish to insist upon the method of "growing the bacilli," as it were, in the warm chamber for from twenty-four to forty-eight hours, and then re-examining the sputa in doubtful cases, as Nuttall[1] demonstrated beyond a doubt that the tubercle bacillus will multiply in the sputum itself at a certain temperature. The value of this observation is obvious, and I have repeatedly been able to demonstrate their presence in this manner when it was impossible to detect them in the fresh sputum. The centrifugal machine in such cases is also useful and yields valuable results, the probabilities of finding the bacilli when present in small number being very much increased.

In the examination of tubercular sputa the fine caseous particles previously described (page 342) should be carefully sought for, as they contain the largest number of bacilli. In their absence reliance should be placed upon the examination of a large number of preparations.

If but few bacilli are present, the following procedure may also be employed: about 100 c.c. of sputum are boiled with double the amount of water, to which from six to eight drops of a 10 per cent. solution of sodium hydrate have been added, until a homogeneous solution has been obtained, water being added from time to time to allow for evaporation. The mixture is then centrifugated or set aside for twenty-four to forty-eight hours and examined for tubercle bacilli and elastic tissue. Or, the following procedure, suggested by d'Arrigo and Stampacchia, may be employed: Four or five sputum masses are placed in a test-tube and covered with Ranvier's acid alcohol (70 per cent. alcohol, containing 1 per cent. of concentrated hydrochloric acid), so that this fills about two-thirds of the tube.

[1] Nuttall, Johns Hopkins Hosp. Bull., 1891.

The mixture is well shaken and is kept, stoppered with cotton, for twenty-four hours at 37° C. or for three hours at 50° C. The acid alcohol destroys the mucus and fixes the cells and bacilli, which sink to the bottom. It is claimed that in a sediment prepared in this manner it is possible to demonstrate the tubercle bacilli even after several years.

If, notwithstanding the fact that all due precautions have been taken, no bacilli can be demonstrated in the sputum, and the clinical history and the physical signs are indefinite or negative, the probabilities are that we are dealing with a benign process. From an examination of the sputa alone in such cases it is utterly impossible to reach a definite conclusion. When the amount of sputum, moreover, is small and contains but little pus, the absence of tubercle bacilli in doubtful cases is less suggestive of the absence of tubercular disease than in cases in which the sputum is more abundant and mucopurulent.

Only two bacilli are likely to be mistaken for the tubercle bacillus, viz., the bacillus of leprosy and the smegma bacillus. All three are characterized by the difficulty with which they take up basic dyes, and the great tenacity with which these are retained when once stained, even upon treatment with mineral acids. This peculiarity has been quite generally referred to the presence of fat in the bacilli, but it appears from more recent researches that the chitin or chitinous substances in the bodies of the tubercle bacilli are primarily concerned in the reaction (Helbing[1]). Sata,[2] moreover, has shown that other bacteria, such as the anthrax bacillus, the bacillus of glanders, the staphylococcus aureus, etc., give a fat reaction which is as intense as that of the tubercle bacillus, while these organisms are not in the least resistant to the action of acids when stained.

That confusion should arise in the differentiation between the tubercle bacillus and the *bacillus of leprosy* is very unlikely. More important is the *smegma bacillus*, which is now known to occur at times upon the tonsils, the tongue, and in the tartar of the teeth of perfectly healthy individuals. In sputum coming from the lungs it has been observed by Pappenheim,[3] Fränkel,[4] and others. To Pappenheim we are indebted for a method by which we are enabled to differentiate such cases from tuberculosis. This is essentially based upon the greater ease and rapidity with which the smegma bacillus is decolorized by means of fluoresceïn-alcohol, as compared

[1] C. Helbing, "Erklärungsversuch f. d. specifische Färbbarkeit d. Tuberkelbacillen," Deutsch. med. Woch., 1900, V. B. p. 133.

[2] Sata, "Ueber d. Fettbildung durch verschiedene Bakterien," etc., Centralbl. f. allg. Path. u. path. Anat., 1900, Nos. 3, 4.

[3] A. Pappenheim, "Befund v. Smegmabacillen im menschlichen Lungenauswurf." Berlin. klin. Woch., 1898, No. 37.

[4] A. Fränkel, "Einige Bemerkungen über d. Vorkommen v. Smegmabacillen im Sputum," Ibid., 1898, p. 880.

with the tubercle bacillus. As the other methods which have hitherto been in use in the clinical laboratory do not permit of differentiation between the two organisms, I have given Pappenheim's method the first place, but have retained the others also. They may be employed as heretofore, unless special reasons exist for eliminating the smegma bacillus, the occurrence of which in the sputum must after all be regarded as a medical curiosity. In the examination of urinary deposits, however, in which the smegma bacillus is far more commonly seen, these older methods are not applicable (see Urine).

Methods of Staining the Tubercle Bacillus.—1. *Pappenheim's Method.*[1]—A drop of the sputum—or, if the cheesy particles described above, are present, one of these—is spread in a thin layer between two cover-glasses. These are then drawn apart, dried in the air, and fixed by being passed three times through the flame of a Bunsen burner or an alcohol lamp. Larger quantities of the sputum may also be employed, and are spread upon slides and examined in the same manner, a drop of immersion oil being placed directly upon the dried and stained preparation. The specimens are covered with a few drops of carbol-fuchsin solution and heated to the boiling-point. The solution is composed of 1 part of fuchsin, 100 parts of a 5 per cent. solution of carbolic acid and 10 parts of absolute alcohol. The excess of the staining fluid is drained off, when the preparations are immersed from three to five times in Pappenheim's solution, care being taken to let the fluid drain off slowly after each immersion. The reagent consists of 1 part of corallin (rosolic acid) in 100 parts of absolute alcohol, to which methylene-blue is added to saturation. This mixture is further treated with 20 parts of glycerin, and is then ready for use. The specimens are finally washed in water, dried between filter-paper, and mounted in balsam or oil of cedar. A $\frac{1}{12}$ oil immersion lens is very convenient, but not a necessity, as the organisms are seen quite readily with lower powers, such as Zeiss' DD, Leitz' 7, or Bausch and Lomb's $\frac{1}{6}$ or $\frac{1}{8}$, with a correspondingly high eye-piece.

2. *Gabbet's Method.*—The dried and fixed preparations are covered for two minutes with the carbol-fuchsin solution described above, and are immediately transferred, without washing, to a solution composed of 2 parts of methylene-blue in 100 parts of a 25 per cent. solution of sulphuric acid, in which they remain one minute. They are then washed in water and mounted.

Instead of staining with the carbol-fuchsin by the cold method, as just described, one can also use heat. To this end, the cover-glass or slide specimen is covered with the fuchsin solution and held over a small flame, so that the stain just barely simmers. The heating is continued for a minute or two, new reagent being added if evaporation should proceed too far. The process is then continued as

[1] Pappenheim, loc. cit.

described. Some investigators prefer to immerse the specimens in the carbol-fuchsin solution for twenty-four hours, but there is no material advantage to be gained in this way.

It has recently been suggested by Pagani[1] to use lactic acid instead of sulphuric acid, in order to avoid a too energetic decolorization. He claims that excellent results are obtained if the second solution of Gabbet is replaced by one of the following formula: water, 50 c.c.; alcohol, 50 c.c.; lactic acid, 2.5 grammes; and methyl-blue to saturation. The cover-glass specimens or slides are immersed in this solution for from fifteen to twenty seconds while gently agitating.

Gabbet's method of staining is very convenient, and is the one most generally employed. The smegma bacillus, however, is also stained.[2]

3. *The Weigert-Ehrlich Method.*—Dried specimens are prepared, and stained for twenty-four hours with a solution of fuchsin in anilin-water, by floating upon the surface. The staining fluid is prepared as follows:

A small test-tube full of water is shaken with about twenty drops of pure anilin oil (1 : 20), and after standing for a few minutes filtered through a moistened filter. To this solution a few drops of a concentrated alcoholic solution of fuchsin or of methyl-violet are added until the mixture becomes slightly cloudy—*i. e.*, until a metallic lustre is noted on the surface. After twenty-four hours the preparations are washed with water in order to remove an excess of staining fluid. They are then immersed for several seconds in a dilute solution of nitric or hydrochloric acid (1 : 6, 1 : 3, or 1 : 2), and washed again with water or with absolute alcohol. At this time the specimens should have a faintly red or violet color. They are then dried between layers of filter-paper or in the air, and mounted as usual.

If it is desired to use a counter-stain, Bismarck-brown, vesuvin, or methylene-blue in watery solutions may be used for the purpose. Into such a solution the specimen is placed after treatment with nitric acid and washing in water. It remains for about two minutes, and is then washed, dried, and mounted as above.

4. *Ziehl-Neelsen's Method.*—A mixture of 90 parts of a 5 per cent. solution of carbolic acid and 10 parts of a concentrated alcoholic solution of fuchsin is used. The procedure is the same as that described under the Weigert-Ehrlich method. It is usually not necessary to stain the preparations for twenty-four hours, however, and as a rule it is sufficient to place a few drops of the staining fluid upon the preparation and to heat over the free flame as described when the specimen is decolorized as before. In this manner excellent results may be obtained in a few minutes.

[1] Pagani, Ref. in Centralb. f. Path. u. path. Anat., 1901, vol. xii. p. 323.

[2] Fränkel, Berlin. klin. Woch., 1884, vol. xxi. p. 195; and Deutsch. med. Woch., 1887, vol. xvii. p. 552.

Stained according to one of these methods, the bacilli appear as rods, measuring about 1.5–3.5 μ in length by 0.2 μ in breadth (Plate XVII., Fig. 1). Much larger specimens may, however, also be seen, up to 11 μ in length. The shortest forms are commonly straight; the common types are usually slightly curved. They may occur joined in chains of two or three, and branching forms have also been observed. Occasionally one may see a couple of organisms, each bent to a crescent, linked in the form of the letter S. Very commonly they are beaded, and it is possible to make out from 1 to 8 clear spaces in an organism which are separated by round or rod-shaped granules, which are deeply stained and appear to lie in a lightly staining capsule. The small hyaline bodies were once regarded as spores, but it is more likely that they are vacuoles. Sometimes bacilli are seen which have club- or knob-shaped swellings at the extremities. These enlargenents likewise have been viewed by some as spores, while others look upon them as products of degeneration. When present in large numbers, they are often seen in clumps, as though the bacilli had been agglutinated side by side, but in every specimen isolated organisms are also found scattered through the field; or two and three are found together.

Cultivation of the Tubercle Bacillus.—The cultivation of the tubercle bacillus is best accomplished on blood-serum or glycerin-agar (agar with 6 per cent. of glycerin added) at a temperature of 37° or 38° C. Below 30° C. and at a temperature higher than 42° C. the organism does not grow. Primary inoculation from the tissue should be made on blood-serum, as the bacillus usually does not grow on glycerin-agar when this is inoculated directly from the tubercular focus. Subcultures, however, grow readily on glycerin-agar and more rapidly than on blood-serum. The individual colonies appear like small dry scales, which gradually coalesce and form a wrinkled film of a dull whitish color. Older cultures present a brownish or grayish-brownish color. An adequate idea may be formed of the growth of the organism after from two to three weeks. Sunlight rapidly kills the tubercle bacillus.

The number of bacilli which may be found in a sputum varies greatly, and while in general it may be said that it is in direct ratio to the intensity of the disease, and may thus be considered of prognostic value, too much reliance should not be placed upon this statement, as in acute miliary tuberculosis, and in cases that have gone to the formation of cavities, the number may be small or they may be absent altogether. In an incipient case, on the other hand, in a little mucoid sputum the number may be large. If the number of bacilli steadily decreases in a series of examinations at intervals sufficiently long, the patient may be regarded as improving, but here the constitutional symptoms and local signs give much more accurate information.

If on repeated examination large numbers of tubercle bacilli are found, the disease has in all probability advanced to cavitation (Brown).

In tabulating the number of tubercle bacilli in reports one may adapt Gaffky's scheme, modified by L. Brown as follows ($\frac{1}{12}$ oil immersion; ocular 1; B. & L.).

1. Only 1–4 in a whole preparation.
2. Only 1 bacillus on an average in many fields.
3. Only 1 bacillus on an average in each field.
4. 2–3 bacilli on an average to each field.
5. 4–6 bacilli on an average to each field.
6. 7–12 bacilli on an average to each field.
7. 13–25 bacilli on an average to each field.
8. About 50 bacilli on an average to each field.
9. 100 or more bacilli on an average to each field.
10. Enormous numbers on an average to each field.

An attempt has been made to attach prognostic significance to form and grouping of the tubercle bacilli in the sputum. To judge from the experience gathered at Saranac, it appears that virulent and attenuated forms of tubercle bacilli possess practically the same morphology and that short bacilli usually represent a younger growth. Arrangement of the bacilli in clumps is more apt to be found in the severer cases, but may occur in all (Brown).

Of the variations in number and form of the tubercle bacilli during treatment with Koch's tuberculin it is unnecessary to speak at this place, as the prognostic significance attaching to such variations is questionable.[1]

The Diplococcus Pneumoniæ.—In doubtful cases the sputum may be examined for the Diplococcus pneumoniæ, and it may be accepted at the present time that its presence in a given case, providing that the clinical history and the physical signs point to a pneumonia, renders the diagnosis of acute *croupous* pneumonia very probable.

Method.—Cover-glass specimens, prepared as indicated above, are placed for one or two minutes in a 1 per cent. solution of acetic acid; they are then removed, the excess of acetic acid is drawn off by means of a pipette, when they are allowed to dry in the air; they are subsequently placed for several seconds in saturated anilin-water and gentian-violet solution, washed in water, and examined. Rod-shaped diplococci (Plate XVII., Fig. 2), surrounded by a capsule, which latter is considered the characteristic feature of this organism, will be seen in cases of acute croupous pneumonia.[2]

The **bacillus of influenza** has already been considered in Chapter

[1] F. Fischel, Unters. über d. Morphol. u. Biol. d. Tuberculose, Erregers, 1895. Gaffky, Mitth. aus d. Kais. Gesundh. Anz., vol. xi. p. 126; L. Brown, Jour. Am. Med. Assoc., 1903, vol. xl. p. 514.

[2] Fränkel, Zeit. f. klin. Med., 1886, vol. ii. p. 437. Weichselbaum, Wien. med. Woch., 1886, vol. xxxix. pp. 1301, 1339, 1367.

I. (page 122). In the sputum it is frequently associated with pyogenic cocci and pneumococci.

In *whooping-cough* protozoa have been observed by Deichler and Kurloff; their observations have not been confirmed, however, and other observers attribute the disease to the presence of bacteria. Among these may be mentioned Affanasiew, Ritter, Czaplewski, Hensel, Koplik, and others. All these investigators claim to have isolated from the sputum of whooping-cough a micro-organism, which they regard as the cause of the disease. Whether or not Affanasiew's bacillus is identical with Ritter's diplococcus and with the pole-bacillus of Czaplewski, Hensel, and Koplik[1] is, however, not clear. **Koplik's organism** is extremely minute, measuring from 0.8 μ to 1.7 μ in length by 0.3 μ to 0.4 μ in breadth. When stained with Löffler's blue it has a finely punctate appearance, like the diphtheria bacillus. In pure culture it is not decolorized by Gram's method. It is anaërobic as well as aërobic, and is apparently not motile. To isolate it from the sputum, it is best to obtain some of the grayish-white pellets which are expectorated during the convulsive stage. In these, small particles will be seen, resembling scales of dandruff. Such particles are isolated and planted first on hydrocele fluid, in order to obtain the crude culture. Later the organism may be grown in bouillon, on agar, gelatin, etc. On Löffler's serum a whitish growth is obtained which closely simulates that of the diphtheria bacillus. The organism is pathogenic for mice, particularly after intraperitoneal inoculation, but it does not produce whooping-cough in the lower animals.

The Smegma Bacillus.—In a few isolated cases the smegma bacillus has been encountered in the sputum, and, as I have already stated, the same organism may normally be present in the saliva, the coating of the tongue, the tartar of the teeth, etc. Like the tubercle bacillus, it resists the decolorizing action of acids when once stained, and may hence be confounded with it unless special precautions are observed (page 357).

Rabinowitch[2] recently succeeded in cultivating from the sputum of a case of pulmonary gangrene an organism which is either identical with the smegma bacillus or closely allied to it; she gives the following account of its cultural characteristics: on glycerin-agar, after twenty-four to forty-eight hours the organism forms grayish-white, lustrous colonies of the size of the head of a pin, which gradually coalesce to a whitish, cream-like coating. On further growth the lustre disappears, the surface appears dry, the coating becomes wrinkled and assumes a yellowish color. Still later,

[1] E. Czaplewski u. R. Hensel, "Bacteriol. Untersuchungen bei Keuchhusten," Deutsch. med. Woch., 1897, p. 586. H. Koplik, "The Bacteriology of Pertussis," Johns Hopkins Hosp. Bull., 1898, p. 79.

[2] L. Rabinowitch, "Befund v. säurefesten tuberkelbacillenähnlichen Bakterien bei Lungengangrän," Deutsch. med. Woch., 1900, No. 16.

when kept at the temperature of the room it turns to a deep orange. The organism is non-motile. It occurs in the form of little rods, which in older cultures manifest a tendency to the formation of long threads. In gelatin stab-cultures small colonies appear along the line of the puncture, which are separated from each other. On the surface a thickish, white, lustrous coating develops, which gradually turns orange. The gelatin is not liquefied. On potato the cultures form a moist, gray coating after two or three days. Bouillon remains clear, but on the surface a wrinkled membrane appears; at the same time a disagreeable odor develops, and a marked indol reaction is then obtained. When injected as such the organism was not pathogenic for guinea-pigs, while inoculation together with sterile butter produced changes identical with those obtained by the same observer in the case of an acid-resisting bacillus which has repeatedly been found in butter. Unlike Pappenheim's organism, the bacillus which was isolated by Rabinowitch was not decolorized by Pappenheim's method. Nevertheless, she regards the two as identical, and looks upon similar acid-resisting bacilli which have been obtained from butter, manure, and various grasses, as closely related organisms.

The Typhoid Bacillus.—It has been conclusively shown that the typhoid bacillus can be present in the sputum of typhoid patients, especially if there is a coëxistent bronchitis or pneumonia.[1]

The **plague bacillus** is seen in the sputum in enormous numbers in cases of the pneumonic type of the disease. By direct observation, however, it may not be recognized immediately, and it is best in every case to resort to culture as well (see page 181.) The organism may be found in the sputum on the first day of the disease.

Actinomycosis of the lungs may at times be diagnosed from the presence of the characteristic granules and thread-like formations in the sputum. Up to and including the 6 cases reported by Ewing[2] in 1902, there are records of 100 American cases.

The organism in question (Fig. 88) the *streptothrix actinomycotica* or *ray fungus* probably belongs to the species cladothrix and occupies a unique position among the pathogenic bacteria. Infection in man and animals (cattle and pigs) possibly occurs through ears of barley or rye, a supposition, with which the observation accords that the disease frequently begins in the autumnal months.

In the pus derived from ulcerating actinomycotic tumors, in the sputum in cases of pulmonary actinomycosis, and also in the feces when the disease has attacked the intestines, yellow granules will be observed, measuring from 0.5 to 2 mm. in diameter. If such a granule is examined microscopically, slight pressure being applied to the cover-glass, it will be seen to consist of numerous

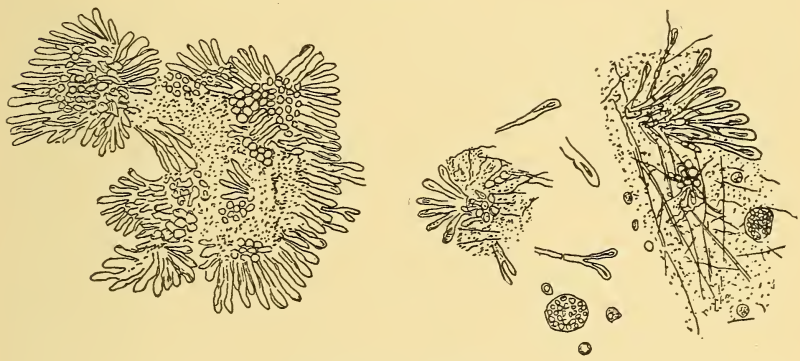
[1] M. W. Richardsen, Boston Med. and Surg. Jour., Feb. 5, 1903.

[2] W. G. Ewing, Johns Hopkins Hosp. Bull., 1902, vol. xiii. J. Ruhräh, Annals of Surg., 1899, vol. xxx. (analysis of 62 cases).

threads which radiate from a centre in a fan-like manner and present club-shaped extremities.

The organism may be demonstrated in the following manner: dried cover-glass preparations are stained for five to ten minutes with a saturated anilin-water and gentian-violet mixture (see page 146), when they are rinsed in normal salt-solution, dried between filter-paper, and transferred for two or three minutes to a solution of iodo-potassic iodide (1 : 100 or 1 : 50). They are then again dried between layers of filter-paper, decolorized in xylol-anilin oil

FIG. 88.



Actinomyces. (MÜSSER.)

(1 : 2), washed in xylol, and mounted in balsam. The mycelium assumes a dark-blue color.[1]

Non-pathogenic Organisms.—Of the non-pathogenic microorganisms which may be observed in sputa little is known.

Oïdium albicans may be seen in children, and is usually derived from the mouth.

Of other fungi which are occasionally observed, there may be mentioned the Aspergillus fumigatus and Mucor corymbifer. Saccharomyces has been seen in pus from pulmonary abscesses. **Sarcina pulmonalis** has been found at times, and especially in the so-called mycotic bronchial props occurring in putrid bronchitis. They are usually smaller than the Sarcinæ ventriculi, but larger than those observed in the urine; they present the characteristic form of the latter. Various other bacilli and micrococci, in addition to those mentioned, are also found in the sputa in large numbers, but have not been closely studied, excepting the pus-organisms, which may almost always be demonstrated.

Crystals.—Of crystals which may occur in sputa, it will be necessary to consider briefly the crystals of Charcot-Leyden, hæmatoidin,

[1] R. Paltauf, Sitzungsber. d. K. K. Gesellsch. d. Aerzte Wien, 1886.

cholesterin, margarin, tyrosin, calcium oxalate, and triple phosphates.

Charcot-Leyden Crystals.[1]—These crystals were discovered in the sputa of patients suffering from bronchial asthma, and were supposed to stand in a causative relation to the disease. This view, however, has been abandoned, and it is now known that they may occur in other diseases as well. But while their presence is almost constant in true bronchial asthma at a time when Curschmann's spirals can also be demonstrated in the sputa, they are only exceptionally met with in other diseases, such as acute and chronic bronchitis, phthisis, etc. They were formerly regarded as identical with *Böttcher's sperma crystals*, but modern research has shown that this is not the case. They are straight hexagonal double pyramids, and appear under the microscope as flattened needles of variable size (Fig. 71). Some attain a length of from 40 μ to 60 μ, while others are scarcely visible even with a comparatively high power of the microscope. They show a feeble, positive double refraction, and have but one optical axis, while the sperma crystals are biaxial and strongly double refracting. Their behavior to solvents is essentially the same as that of the sperma crystals, but they differ from these in their insolubility in formol. They are colored yellow with Florence's reagent, while the sperma crystals are stained a bluish black. Very curiously the appearance of Charcot-Leyden crystals is closely associated with the presence of eosinophilic leucocytes, and they have hence not inaptly been termed *leucocytic crystals*. They may in fact originate within the cells. In true bronchial asthma it is not uncommon to find microscopical preparations of the sputum literally studded with eosinophilic leucocytes and free granules. Outside the sputum they are also found in the blood in myelogenous leukæmia, and in the stools in association with animal parasites. They readily form in both normal and abnormal red bone-marrow, and excellent specimens may be obtained for purposes of demonstration if a piece of a rib is allowed to remain exposed to the air for a few days. The marrow then usually contains large numbers. The crystals also form in decomposing viscera in general, and at times form a complete covering of old anatomical preparations. Their occurrence may be regarded as evidence of retrogressive changes in the cellular elements of any organ. Of the relation which they bear to the eosinophilic leucocytes, with which they are so constantly associated, nothing is known. The Charcot-Leyden crystals can be stained with the triacid stain, with thionin, with the eosinate of methylene-blue, and others.

Hæmatoidin crystals may be observed in the sputa following ex-

[1] Leyden. Virchow's Archiv, 1872, vol. liv. p. 324. Schreiner, Liebig's Annal., 1878, vol. cxciv. p. 68. Cohn, Centralbl. f. allg. Path. u. path. Anat., xol. x. p. 940. Brown, Phila. Med. Jour., 1898, p. 1076.

travasations of blood into the lung. They frequently occur in the form of ruby-red columns or needles; amorphous granules, however, are also seen, enclosed in the bodies of leucocytes, in which case they are probably always indicative of a previous hemorrhage, while the needles are generally observed when an abscess or empyema has perforated into the lungs. The substance is derived from blood-pigment, and is now known to be identical with bilirubin.

Cholesterin crystals are at times seen in the sputa in cases of phthisis, pulmonary abscess, and, in general, whenever old accumulations of pus have entered the lung from a neighboring organ. They are readily recognized by their characteristic form and chemical properties (see Feces, page 282).

Fatty acid crystals are frequently observed in cases of putrid bronchitis and gangrene of the lung, and also in cases of bronchiectasis and phthisis. They occur in the form of single needles or groups of needles, which are long and pointed. They are easily soluble in ether and hot alcohol; insoluble in water and acids. Chemically, they are probably composed of the higher fatty acids, such as palmitic and stearic acids.

Tyrosin crystals have been observed in cases of putrid bronchitis, perforating empyema, etc. **Leucin** is likewise probably always present, occurring in the form of highly refractive globules. For the recognition of these bodies, particulary of tyrosin, a chemical examination should always be made, as crystals of the soaps of fatty acids have frequently been mistaken for those of tyrosin (see Urine).

Calcium oxalate crystals are rarely seen. Fürbringer observed them in large numbers in a case of diabetes, and Unger found them in a case of asthma. They are readily recognized by their envelope-form, but they occur also in amorphous masses. They are soluble in mineral acids; insoluble in water, alkalies, organic acids, alcohol, and ether.

Triple phosphate crystals also are rarely seen, but may occur in cases of perforating abscesses, etc. They are recognized by their coffin-lid shape and the readiness with which they dissolve in acetic acid.

THE PNEUMOCONIOSES.

Anthracosis.—To some extent particles of carbon may be found in the sputum of almost every individual, and especially in smokers. The expectoration in such cases is of a pearl-gray color, and is brought up in larger or smaller masses, especially in the morning upon rising. Larger amounts are noted in miners and in those who are brought into close contact with coal-dust. Microscopically, particles of carbon and epithelial cells, especially of the alveolar type, as well as leucocytes loaded with the pigment, are seen.

Siderosis.—In siderosis the sputum presents a brownish-black

color and contains cells enclosing particles of ferric oxide. These may be readily recognized by treating the preparation with a drop of ammonium sulphide or potassium ferrocyanide solution in the presence of hydrochloric acid, when a black color on the one hand or a blue color on the other is obtained in the presence of iron.

Chalicosis.—In chalicosis silicates are found in the sputa.[1]

Stycosis.—This condition was first described by A. Robin in a man, aged seventy, who from his seventeenth year suffered from cough and frequent attacks of diarrhœa, and whose condition at various times had been diagnosed as phthisis pulmonalis et intestinarum, although tubercle bacilli could not be demonstrated. The patient died from acute pericarditis complicating an attack of acute mono-articular rheumatism. Post mortem the lungs were found perfectly normal; the bronchial and anterior mediastinal glands, as well as the mesenteric glands, however, were completely solidified and composed almost wholly of calcium sulphate. The man, it was then found, had been working in plaster of Paris all his life, and the symptoms observed—viz., cough, expectoration, and diarrhœa—Robin is inclined to attribute to the pressure of the solidified glands upon the bronchi and intestines.

CHEMISTRY OF THE SPUTUM.

In addition to the substances described, sputum contains certain albumins, volatile fatty acids, glycogen, ferments, and various inorganic salts.

Among the albumins which have been observed in sputa may be mentioned serum-albumin, and especially mucin, which is often present in large amounts. In pneumonic and purulent sputa albumoses also have been found.

In order to demonstrate the presence of serum-albumin the sputa are treated with dilute acetic acid, when the filtrate is tested with potassium ferrocyanide, as described in the chapter on Urine. Serum-albumin is, of course, found in notable quantities in cases of œdema of the lungs. Especially interesting is the *albuminous expectoration* which at times follows thoracentesis. The amount of sputum usually varies between 200 and 900 grammes, but may be much larger and may reach 2000 c.c. or even more. Occasionally it begins before the tapping is completed or immediately after. More commonly, however, an interval varying from five minutes to one or two hours elapses before the expectoration begins. Its duration is variable. Sometimes it lasts only a few minutes, more often an hour or two, and in rarer cases a whole day or two. The condition is probably due to œdema of the lungs.[2]

[1] Betts, Chalicosis Pulmonum," Jour. Am. Med. Assoc., 1900, No. 2.

[2] In the United States cases of albuminous expectoration following thoracentesis have been reported by Pepper, Allen, Pateck, and Riesman. See especially the paper by Riesman, in which a full account of the literature is given. Am. Jour. Med. Sci., April, 1902, p. 620.

The volatile fatty acids contained in sputa may be obtained by diluting with water, acidifying with phosphoric acid, and distilling, when the distillate is further examined as described in the chapter on Feces. Acetic, butyric, propionic, and capronic acid has been found.

The fats and fixed fatty acids are extracted from the residue with ether, and shaken with a solution of sodium carbonate in order to transform them into their sodium salts, when the ether is decanted and evaporated, leaving the soaps behind.

Glycogen has repeatedly been demonstrated in sputa, and may be detected by Ehrlich's method (see page 138).

The sputa of gangrene of the lung and putrid bronchitis have been shown to contain a ferment resembling trypsin. In order to test for this ferment, the sputa are extracted with glycerin ; the examination is then continued as described in the chapter on the Examination of Cystic Contents.

The myelin granules, as I have already indicated, consist largely of protagon, lecithin, and cholesterin.

The following are the inorganic salts which may be demonstrated in the sputum : sodium and magnesium chloride, phosphates of the alkalies and the alkaline earths (viz., calcium and magnesium), calcium and sodium sulphate and carbonate, phosphate of iron, and silicates.

CHAPTER VII.

THE URINE.

GENERAL CONSIDERATIONS.

This is not the place to enter into a discussion of the various hypotheses which have been advanced to explain the manner in which waste-material is removed from the body through the kidneys. It will suffice to state that while the water and mineral constituents of the urine in part at least undoubtedly pass into the uriniferous tubules by a process of transudation, a selective glandular activity of the cells lining the convoluted tubules and the loop of Henle appears to be necessary for the elimination of the most important organic constituents.

As the physical characteristics of the urine, as well as its chemical composition, are influenced not only by the age and sex of the individual, but also by the character of the food ingested, the process of digestion, exercise, climate, temperature, race, etc., it is apparent that a quantitative analysis of any one urine, or even average figures, can give only an approximate idea of its composition. The reader is accordingly referred for information to the special paragraphs concerning the variations in the individual constituents observed in health. It is important, however, to note that, notwithstanding the fairly wide variations here observed, the composition of the blood, as pointed out in a previous chapter, remains quite constant, showing the perfect manner in which the nervous system through the kidneys guards against an undue accumulation of what may be termed normal waste-products in the blood, and in virtue of which abnormal substances are also immediately eliminated. Moreover, as will be pointed out later on, a perfect mechanism appears to exist which prevents an undue accumulation of material in the blood that can hardly be regarded as waste. The presence of an amount of sugar in the blood exceeding 6 pro mille, for example, appears to be invariably followed by glucosuria, and the introduction of excessive quantities of sodium chloride similarly and almost immediately leads to an elimination of the excess.

GENERAL CHARACTERISTICS OF THE URINE.

Appearance.

Normal urine, just voided at an ordinary temperature, is either perfectly clear or but faintly cloudy, owing to the fact that the acid and normal salts present are all soluble in water. It may be stated, as a general rule, that whenever a urine *freshly passed* presents a distinct cloudiness some abnormality exists.

When allowed to stand for a time a light cloud develops, which gradually settles to the bottom, constituting the so-called *nubecula* of the ancients. Examined under the microscope this is found to contain a few round, granular cells, somewhat larger than normal leucocytes, the so-called *mucous corpuscles*, and a few pavement-epithelial cells, derived from the bladder or genital organs. Chemically the nubecula probably consists of traces of mucus.

When kept for twenty-four hours at an ordinary temperature crystals of uric acid are frequently observed in addition to the above elements, usually presenting the so-called whetstone-form. If, however, the temperature at which the urine is kept approaches the freezing-point, the entire volume becomes cloudy, owing to precipitation of acid urates, as these are much less soluble in cold than in warm water; on standing they gradually settle to the bottom of the vessel, and form what is known as a *sediment*, while the supernatant fluid again becomes clear.

If kept still longer exposed to the air, at the temperature of the room, the entire volume of urine again becomes cloudy, owing to a diminution of its normal acidity, the result being a precipitation of ammonio-magnesium phosphate, calcium phosphate, and still later, when the urine has become alkaline, of ammonium urate.

Gradually a heavy sediment, containing these salts in addition to the constituents of the primitive nubecula, forms at the bottom of the vessel; the supernatant fluid, however, remains cloudy. On microscopical examination it will be seen that this cloudiness is due to the presence of enormous numbers of bacteria.

The changes which take place in a normal urine when allowed to stand at ordinary temperature may be tabulated as follows:

(1) Urine clear, no sediment—reaction acid.

(2) Urine slightly cloudy, owing to development of the nubecula —reaction acid.

Nubecula { Mucous corpuscles, Pavement-epithelial cells.

(3) Urine clear, the nubecula has settled—reaction acid.

Sediment { Mucous corpuscles, Epithelial cells, Uric acid crystals, A few bacteria.

(4) Urine cloudy, owing to the precipitation of phosphates—reaction faintly acid.

(5) Urine cloudy, owing to the presence of bacteria—reaction alkaline.

Sediment { Bacteria, Mucous corpuscles, Epithelial cells, Triple phosphates, Tri-calcium phosphate, Ammonium urate.

Color.

The color of normal urine may vary from a very light yellow to a brownish red, the particular shade depending essentially upon the specific gravity, becoming lighter with a diminishing, and darker with an increasing density. Pathologically the same rule holds good, except in diabetes, in which a very high specific gravity is generally associated with a very light color. The reaction of the urine also exerts a marked influence upon its color, an acid urine being more highly colored than an alkaline urine, which can be readily demonstrated by allowing a specimen of acid urine to become alkaline, and by treating an alkaline urine with dilute hydrochloric or acetic acid. At the same time it may be said that every urine darkens slightly on standing, the reaction remaining acid.

The various shades observed in normal urines may be grouped under the following headings :

1. Pale urines vary from a faint yellow to a straw color.
2. Normally colored urines are of a golden or an amber yellow.
3. Highly colored urines present a reddish-yellow to a red color.
4. Dark urines vary between brownish red and reddish brown.

As these shades may occur in both normal and pathological urines, definite conclusions cannot, as a rule, be drawn from mere inspection. A very pale urine indicates simply an excess of water, which may be normal, but may also occur in such diseases as chronic interstitial nephritis, diabetes mellitus, diabetes insipidus, hysteria, and the various anæmias ; it is further seen during convalescence from acute febrile diseases, while a highly colored urine, though also occurring in health, may indicate the existence of a febrile process. It may be stated, as a general rule, that a pale urine always excludes the existence of a febrile disease of any severity, and that the continued secretion of a very pale urine is usually associated with a certain degree of anæmia.

The normal color of the urine is probably owing to the presence of several pigments, which are most likely closely related to each other and to hæmatin.

In addition to these colors others may be observed at times which are either pathological or accidental—*i. e.*, due to the presence of cer-

tain drugs. The former are, on the whole, of greater importance to the physician than those mentioned above, as more definite conclusions can be drawn from their presence. Most important among such pathological pigments are those due:

1. To the presence of blood-coloring matter. The color in such cases may vary from a bright carmin to a jet black, the exact shade depending upon the quantity of blood-coloring matter present, upon changes that the blood may have undergone either before or after being passed, and also upon the presence of the pigment in solution or contained in red corpuscles.

2. Those due to the presence of biliary coloring matter. The color here varies from a greenish yellow to a greenish brown.

3. A milky-colored urine is observed in cases of chyluria.

Among the accidental abnormalities in color, on the other hand, are those due to the presence of substances like carbolic acid and its congeners, santonin, etc.

As the recognition of the causes of such alterations, normal, pathological, and accidental, largely depends upon a more detailed study of the individual pigments, this subject will be dealt with more fully further on (see Pigments and Chromogens).

Odor.

The odor of the urine is usually of little significance. Normally it resembles that of bouillon, and in some cases that of oysters; it is probably due to the presence of several volatile acids. The odor of urines undergoing decomposition is characteristic and has been termed "the urinous odor of urine," an ill-chosen term, as this odor is always indicative of an *abnormal* condition.

The ingestion of asparagus, onions, oil of turpentine, etc., produces a characteristic odor which is of no significance.

Consistence.

Urine, while normally fluid and but slightly viscid, may in disease acquire a marked degree of viscidity, which becomes especially apparent upon attempting its filtration; the liquid passes through the paper with more and more difficulty, and finally clogs its pores altogether.

Quantity.

The quantity of the urine is normally subject to great variations, the amount eliminated in the twenty-four hours being influenced by that of the fluid ingested, the nature and quantity of the food, the process of digestion, the blood-pressure, the surrounding temperature, sleep, exercise, body-weight, sex, age, etc.

It is easy to understand, then, why figures given by different

observers in different countries should vary considerably. Salkowski, in Germany, thus gives 1500 to 1700 c.c. as the normal amount; v. Jaksch, in Austria, 1500 to 2000 c.c.; Landois and Sterling, in England, 1000 to 1500 c.c.; Gautier, in France, 1250 to 1300 c.c. In the United States I have found an average secretion of from 1000 to 1200 c.c. in the adult male, and 900 to 1000 c.c. in the adult female. It is thus seen that the secretion of urine is greatest in Germany and Austria, where the body-weight and ingestion of liquids are greater than in England, France, and the United States.

Children pass less, but relatively more (considering their body-weight) urine than adults.

Women pass somewhat less than men.

During the summer months, when a larger proportion of water is eliminated through the skin and lungs than in cold weather, less urine is voided. The same occurs during repose, more urine being passed during active exercise, and hence less during the night than during the day.

The amount of urine secreted in the different hours of the day varies greatly, reaching its maximum a few hours after meals. It decreases toward night, and reaches its lowest point in the first hours of the night, after which it begins to rise rapidly until 2 or 3 o'clock in the morning.

The ingestion of large amounts of liquid, of course, increases the daily amount considerably, and 3000 c.c. may be passed under such conditions by an individual in good health, while it may decrease to 800 or 900 c.c. when but little liquid is taken.

After the ingestion of much solid food the secretion of urine is temporarily diminished.

Water containing no salts possesses distinctly diuretic properties, as do also beer, wine, coffee, tea, etc.

The most important medicinal diuretics are digitalis, squill, broom, spirit of nitrous ether, juniper, urea, etc.

Pathologically the amount of urine varies within wide limits. In a given case, moreover, it may be exceedingly difficult to determine whether or not the secretion is within physiological limits. As a general rule, whenever less than 500 c.c. or more than 3000 c.c. are passed some abnormal condition exists, providing all other causes which might lead to the secretion of such an amount can be eliminated.

Clinically we speak of *polyuria* and *oliguria*.

Polyuria.—Polyuria is observed in many diseases, and is present under such varied conditions that a classification is only warrantable upon a hypothetical basis, especially as the causative factors concerned in its production are mostly unknown.

As polyuria is almost invariably associated with diabetes mel-

litus, its presence in any case should always excite suspicion and lead to a proper examination. The quantity of fluid eliminated in diabetes is usually dependent upon the amount ingested. The excretion of a proportionately large amount of fluid, however, does not necessarily follow the ingestion directly, and retention of a large amount may occur; it has been shown, as a matter of fact, that the diabetic patient excretes liquids with greater difficulty than the healthy subject. At the same time it should be borne in mind that the polyuria in diabetes is not necessarily continuous, and that periods during which a normal or even a subnormal amount of urine is observed may alternate with true polyuria. From 2 to 26 or even 50 liters may be passed within twenty-four hours. Intercurrent diseases of a febrile character may modify the quantity very materially and cause the elimination of a normal or subnormal amount.

The cause of the polyuria in diabetes mellitus is unknown. The ingestion of large amounts of liquids, of course, leads to a correspondingly large elimination, and the existing polydipsia could, hence, be made responsible for the polyuria; the latter would thus be the result of an increased stimulation of the thirst-centre, possibly owing to the presence of some abnormal constituent of the blood. The polydipsia, however, may also be the result of a primary polyuria.

The polyuria associated with the resorption of large pericardial, pleural, ascitic, and subcutaneous effusions is more readily understood, although the *primum mobile* may be unknown; it depends in such cases entirely upon the presence of excessive quantities of fluid in the bloodvessels.

A form of polyuria which has been termed "epicritic polyuria" is frequently observed during convalescence from acute febrile diseases, and is of prognostic importance. Its occurrence in a given case is regarded by many as a good omen, especially in typhoid fever; still it must not be forgotten that a polyuria may occur after subsidence of the fever, and be followed by a considerable degree of oliguria, and in some cases may precede death. A polyuria of this kind probably always indicates the elimination of waste-products which had accumulated in the blood during the course of the disease, but it may, at the same time, be due to the presence of retained water.

Second in constancy is the polyuria associated with granular atrophy of the kidneys, constituting one of the most important symptoms of the disease. Cases have been reported in which as much as 10,000 c.c. of urine were secreted in the twenty-four hours; 2000 to 4000 c.c. represent the usual amount in such cases. Polydipsia commonly exists at the same time, and the explanation of the polyuria again becomes a very difficult matter. That generally given is based upon the following considerations:

In granular atrophy of the kidneys large tracts of renal parenchyma are destroyed, the result being a diminution in the area of glandular material, which in itself would lead to a diminished secretion of urine. The coexisting cardiac hypertrophy, however, by raising the blood-pressure in the kidneys, is supposed to counterbalance the renal deficiency and even to lead to an increase in the amount of urine. There appears to be some doubt as to the correctness of such an explanation, however, as the existence of hypertrophy of the left ventricle in the absence of glandular disease of the kidneys by no means leads to a degree of polyuria comparable to that observed in this disease. It is possible that while cardiac hypertrophy in itself may be *one* of the causative factors, still another may be a vicarious action of the sound glandular elements. If such be the correct explanation, the coexisting polydipsia is merely secondary. This, however, can only be regarded as an hypothesis, and the diminished renal secretion associated with a gradually developing cardiac dilatation should not be upheld as an absolute proof of its correctness.

Very curiously, polyuria may occur also in association with multiple myelomata of the bones and the presence of Bence Jones' albumin in the urine. In one of the cases reported by Hamburger,[1] and which I had occasion to study in greater detail from a chemical point of view, 3500 c.c. were voided in the twenty-four hours. The symptom, however, is not constant.

Polyuria, furthermore, has been observed in the most diverse diseases of the nervous system, both functional and organic. It is frequently observed both as a transitory and a permanent symptom in cases of hysteria. Large quantities of a very pale urine are secreted after the occurrence of severe hysterical seizures, but the same may be observed throughout the course of the disease. A similar condition is frequently seen in neurasthenia, migraine, chorea, and epilepsy.

Generally speaking, it may be said that a *paroxysmal* polyuria in nervous diseases is associated with functional derangement, while a *continuous* polyuria appears to be connected rather with true organic changes. It has been observed in certain cases of tabes, cerebrospinal and spinal meningitis, during the first stage of general paresis, in association with tumors involving the medulla, the cerebellum, and the spinal cord, in injuries affecting the central nervous system, in Basedow's disease, etc. Cases of idiopathic diabetes insipidus also should probably be classified under this heading. Enormous quantities of urine may be secreted in this disease, which are equalled only by cases of diabetes mellitus, and may at times reach 43 liters per diem.

[1] L. P. Hamburger, "Two Examples of Bence Jones Albuminosuria associated with Multiple Myeloma," Johns Hopkins Hosp. Bull., Feb., 1901.

Oliguria.—Oliguria is, on the whole, more frequent than polyuria, and is met with in almost all conditions associated with a lowered blood-pressure. First in order stand those cases of cardiac disease in which compensation has failed, whether the cardiac weakness is primary or occurring secondarily to other diseases—*i. e.*, pulmonary, hepatic, and renal.

The oliguria observed in the so-called continued fevers, notably typhoid fever, is probably also referable to cardiac weakness. It should be remembered, however, that a larger proportion of water is eliminated through the skin and lungs than normally, and that a retention of fluids also undoubtedly occurs which is not due to cardiac weakness; still other factors may be concerned in its production.

The oliguria occurring in acute nephritis and in chronic parenchymatous nephritis in all probability depends largely upon mechanical causes, the increased intra-canalicular resistance in the form of desquamated epithelium and tube-casts, as well as the pressure of the exudate upon the bloodvessels obstructing the passage of urine, while the functional activity of the diseased glandular elements is at the same time lowered. Upon mechanical causes, also, depend all those cases of oliguria which are associated with the presence of a stone or tumor pressing upon a portion of the urinary tract.

Oliguria may occur as a nervous manifestation in connection with puerperal eclampsia, lead colic, hysteria, psychic depression, preceding and during epileptic seizures, etc. Whenever there is a diminution in the amount of bodily fluids oliguria is also observed; this is particularly marked in cholera and following severe hemorrhage.

Obstruction to the flow of blood in the vena cava or liver, leading to an increase of venous pressure and a decrease of arterial pressure in the kidneys, likewise results in oliguria, as is seen in atrophic hepatic cirrhosis, acute yellow atrophy, thrombosis of the vena cava and the renal vein, or in cases in which pressure is exerted upon these by tumors, ascitic fluid, etc.

In any case the oliguria may go on to complete anuria, which condition not infrequently precedes death. Anuria may, however, also occur independently of a pre-existing oliguria, as in hysteria.

Specific Gravity.

The specific gravity of normal urine varies between 1.015 and 1.025, corresponding to 1200 to 1500 c.c., viz., the normal amount of urine voided in twenty-four hours. Pathologically, a specific gravity of 1.002 on the one hand and 1.060 on the other may occur, depending upon the amount of solids and fluids present, increasing as the solids increase, the amount of urine remaining the same, and decreasing as the amount of fluid increases, the solids remaining the

same. The specific gravity is thus an index in a general way of the metabolic processes taking place in the body.

The necessity of determining the specific gravity of the total amount of urine voided in a given case, and not that of an individual specimen passed during the twenty-four hours, becomes apparent upon considering the variations which may occur in the quantity of solids and liquids ingested during the day. The ingestion of large amounts of water or beer would, of course, result in the passage of a correspondingly large quantity of urine within the next few hours, containing but a small amount of solids, and hence presenting a low specific gravity. From such an observation it would be erroneous to infer a diminished excretion of solids for the day, as succeeding specimens would in all probability be passed presenting a higher specific gravity. An observation made upon a specimen taken from the collected urine of the twenty-four hours, moreover, can only then convey a correct idea if the total quantity is within the normal limits. If this should not be the case, the volume of the urine passed must first be reduced to the normal and the specific gravity then taken.

Supposing a known quantity of common salt to be dissolved in 1000 c.c. of water, so that the resulting specific gravity is 1.24; by doubling the amount of salt and water the specific gravity would still remain the same, while the amount of salt would actually be twice as large as at first. In order to obtain the specific gravity indicating the actual amount of solids present it would be necessary to concentrate the fluid to 1000 c.c. The specific gravity being inversely proportionate to the amount of fluid secreted, the necessary correction is made according to the following formula:

$$\text{Sp. gr.} = \frac{qd}{N},$$

in which Sp. gr. indicates the specific gravity to be determined, q the amount of urine actually passed, d the specific gravity observed, and N the normal amount of urine—*i. e.*, 1200 c.c.

Example.—A patient has passed 3000 c.c. of urine in the twenty-four hours with a specific gravity of 1.017; this is corrected according to the above formula:

$$\text{Sp. gr.} = \frac{3000 \times 17}{1200} = 1.042.$$

From the specific gravity the amount of solids can be calculated with sufficient accuracy for clinical purposes by multiplying the last two decimal points by 2, the number obtained indicating the amount of solids in 1000 c.c. of urine.

To illustrate the necessity of either indicating the total amount of urine passed within the twenty-four hours, and of taking the specific

gravity from this collected urine, or of correcting the specific gravity as shown above, the following case may be supposed:

A "specimen" of urine is taken, presenting a specific gravity of 1.002; by multiplying the 2 by 2, the person would be supposed to pass 4 grammes of solids in every 1000 c.c. of urine. Had the specific gravity been observed in the total amount of urine passed in the same twenty-four hours, it would have been found to be 1.012, the man having passed 3000 c.c. of urine; by multiplying 12 by 2, 24 grammes of solids would have represented the amount in every 1000 c.c.—*i. e.*, $24 \times 3 = 72$ grammes *in toto*. The same result would have been reached by correcting the specific gravity of 1.012 for the normal amount of urine.

The first calculation then would have indicated a considerable deficit as compared with the second.

The following rules for practice may thus be stated:

1. Whenever the specific gravity *only* is to be indicated in a urinary report it should always be the corrected one; if this is not done, the amount of urine should be stated in every case.

2. The specific gravity should always be taken from a specimen of the collected urine of the twenty-four hours, and never from a specimen *ad libitum*.

From the rule, that the specific gravity of a urine is inversely proportionate to the amount of fluid eliminated, it must follow that whatever causes produce oliguria will also produce a high specific gravity, while all those causes which produce a polyuria will similarly produce a low specific gravity, with the following exceptions:

1. A diminished amount of urine with a lowered specific gravity occurs in many chronic diseases and toward the fatal termination of acute diseases, indicating a defective elimination of solids.

2. The same may be observed in certain cases of œdema.

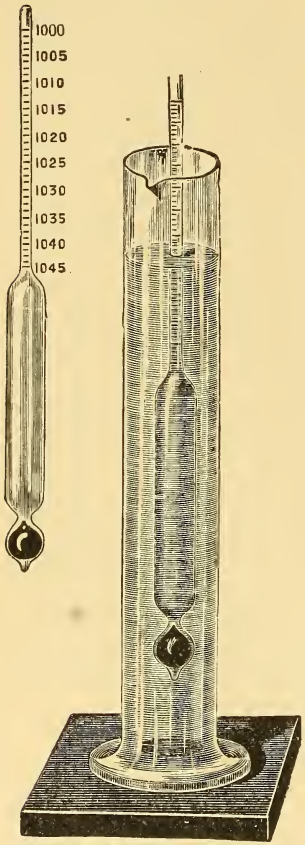
3. Following copious diarrhœa, vomiting, and sweating.

4. A high specific gravity is associated with polyuria in diabetes mellitus.

Unfortunately the determination of the specific gravity and the solids contained in urines does not furnish as valuable information in many cases as would be expected *à priori*. This is largely owing to the fact that the organic constituents of the urine have a lower specific gravity than the inorganic salts, and especially the chlorides, which are usually present in considerable amount. It thus not infrequently happens that the nitrogenous constituents are considerably increased, while the specific gravity is relatively low, owing to the absence or a diminution in the amount of chlorides. In other words, while the specific gravity may be regarded as a fair index of the total amount of solids excreted, its increase or decrease furnishes no information as to the nature of the constituents causing such a change.

Determination of the Specific Gravity.—The specific gravity of the urine is most conveniently determined by means of a hydrometer indicating degrees varying from 1.002 to 1.040. Such instruments, constructed especially for the examination of urine, are termed *urinometers* (Fig. 89). A good instrument should have a stem upon which the individual divisions are at least 1.5 mm. apart, and each division should correspond to 0.5 degree.

Fig. 89.



Urinometer. (W. Simon.)

Urinometers may also be purchased which are provided with a thermometer, a matter of great convenience. Every instrument should be carefully tested by comparison with a *standard* hydrometer.

In order to determine the specific gravity in a given case a cylindrical vessel is nearly filled with urine and the urinometer *slowly* introduced, the reading being taken at the lower meniscus as soon as the instrument has come to rest.

Precautions: 1. The urinometer must be given ample room, and the reading should never be taken when the instrument touches the sides of the vessel, as owing to capillary attraction it is otherwise raised, causing the reading to be too high.

2. The instrument must be perfectly dry and clean before being used, and should never be allowed to "drop" into the urine, as otherwise the weight of the instrument is increased by adhering drops of water, and the reading is too low.

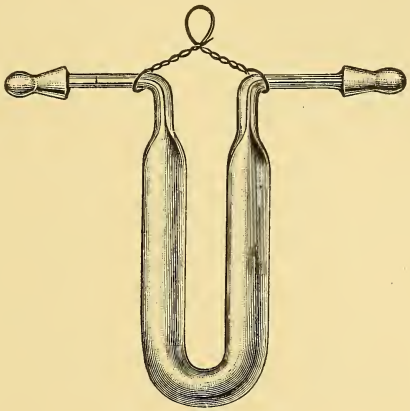
3. Any foam upon the surface of the urine should first be removed by means of a piece of filter-paper, as it interferes with the accuracy of the reading; bubbles of air adhering to the instrument, and thereby elevating it, should be carefully removed with a feather.

4. The specific gravity should always be determined in specimens taken from the twenty-four-hour urine, and corrected according to the formula given above.

5. If the quantity of urine is too small to determine its specific gravity with a urinometer, the following method may be advantageously employed:

About 50 c.c. of urine are measured into a small bottle provided with a ground-glass stopper, or into a pyknometer like the one pictured in Fig. 90, and accurately weighed. The weight of the

FIG. 90.



The pyknometer.

urine divided by its volume gives the specific gravity, which must, however, be corrected for the temperature of the urine. If accuracy is required, such corrections should be made in every case, as the specific gravity increases or decreases by one degree for every three degrees C. above or below the point for which the instrument is registered, viz., 15° C. According to Bouchardat and Mercier, this method is not strictly accurate, and the following table has been constructed by which the proper corrections can be readily made:

Temperature.	Normal urine.	Sugar urine.	Temperature.	Normal urine.	Sugar urine.
0°	0.9	1.3	18°	0.3	0.6
1	0.9	1.3	19	0.5	0.8
2	0.9	1.3	20	0.9	1.0
3	0.9	1.3	21	0.9	1.2
4	0.9	1.3	22	1.1	1.4
5	0.9	1.3	23	1.3	1.6
6	0.8	1.2	24	1.5	1.9
7	0.8	1.1	25	1.7	2.2
8	0.7	1.0	26	2.0	2.5
9	0.6	0.9	27	2.3	2.8
10	0.5	0.8	28	2.5	3.1
11	0.4	0.7	29	2.7	3.4
12	0.3	0.6	30	3.0	3.7
13	0.2	0.4	31	3.3	4.0
14	0.1	0.2	32	3.6	4.3
15	0.0	0.0	33	3.9	4.7
16	0.1	0.2	34	4.2	5.1
17	0.2	0.4	35	4.6	5.5

Example.—Supposing the specific gravity to have been 1.030, at a temperature of 20° C., it would be necessary to add 0.9 to 1.030,

making this 1.0309; at a temperature of 10° C., it would similarly be necessary to subtract 0.5.

Determination of the Solid Constituents.—As indicated above, the amount of solids can be calculated with a degree of accuracy sufficient for clinical purposes by multiplying the last two figures of the specific gravity by 2; the number obtained indicates the amount of solids in every 1000 c.c. of urine. If greater accuracy is required, the following method may be employed:

Five c.c. of urine, accurately measured, are placed in a watch-crystal containing a little dry sand (sand and crystal having been previously weighed); this is placed over a dish containing concentrated sulphuric acid, and under the receiver of an air pump which has been made perfectly air-tight by thoroughly lubricating the ground-glass edge of the bell with mutton tallow and applying the bell with a slightly grinding movement to the ground-glass plate. The receiver is now exhausted and the urine allowed to remain in the vacuum for twenty-four hours, when the bell is again exhausted and left for twenty-four hours longer; at the end of this time the crystal is weighed, the difference between the two weights obtained indicating the amount of solids in 5 c.c. of urine, from which the percentage and total amount are readily calculated.

The slight loss of ammonia which results when this method is employed scarcely affects the accuracy of the result.

REACTION.

The reaction of the twenty-four-hour urine is, as a rule, acid; individual specimens, passed in the course of the same twenty-four hours, may be either alkaline, acid, or amphoteric.

When a mixture of different acids is brought into contact with a mixture of alkalies, the acids combine with the alkalies according to the degree of affinity which exists between them and the amount present of each. Upon the excess of acids over alkalies, and *vice versa*, depends the resulting reaction. If the alkalies are not sufficient in amount to saturate the acids, an acid reaction will result, while an insufficient amount of acid will give rise to an alkaline reaction. The same principle holds good for the acids and alkalies giving rise to the salts present in the urine. As here the alkaline substances are not present in sufficient amount to saturate the acids, which can readily be seen from the following table, the acid reaction of normal urine is explained:

HCl	SO_3	P_2O_5	K	Na	NH_3	Ca.	Mg
10.1265	2.3157	3.0334	2.5830	5.4780	0.5977	0.0405	0.0880
6.3811	1.3315	0.9827	1.5194	5.4780	0.8087	0.0233	0.0843

The figures in the first column indicate the average daily amount

of the inorganic acids and alkalies present in the urine of twenty-four hours, and the figures in the second column their equivalents in terms of sodium, that of phosphoric acid having been estimated as diacid sodium phosphate. From this it is seen that the acid equivalents, 8.6953, exceed the alkaline equivalents, 7.9137, by 0.7816 gramme of sodium. There are present then in the urine, in addition to the normal salts of the monobasic acids, acid salts and especially diacid sodium phosphate, NaH_2PO_4. To the latter the acidity of the urine is due. If, on the other hand, the alkalies exceed the acids in amount, an alkaline urine will result, which may occur physiologically under various conditions.[1]

The so-called amphoteric reaction will be observed when the diacid and neutral sodium phosphates, NaH_2PO_4 and Na_2HPO_4, are present in a certain definite proportion; the urine then changes the color of red litmus paper to blue, and *vice versa*.[2]

A neutral urine is never observed under normal conditions. The presence of a free acid, moreover, is not possible, as it would immediately combine with ammonia, which is constantly being set free in the tissues of the body as ammonium lactate, and is normally transformed into urea.[3]

The question now arises, How does the acidity of the urine result? and What are the ultimate factors that will produce an alkaline and an amphoteric reaction?

These are problems which as yet await a final answer. Our present ideas, however, may be formulated as follows: In the metabolism of the body-tissues acids are constantly produced; chief among these is sulphuric acid, which results from albuminous decomposition, and hydrochloric acid, which at a certain period of digestion is reabsorbed from the stomach. As the alkalinity of the blood is due to neutral sodium phosphate and sodium carbonate, these salts are attacked by the free acids as soon as they enter the blood, the result being the formation of acid salts, and, as the latter diffuse more readily through an animal membrane than alkaline salts, the secretion of an acid urine from the alkaline blood is in part explained. Nevertheless it is impossible to exclude a certain specific action on the part of the glandular elements of the kidneys, as otherwise the secretion of all glands, supposing this to depend upon a process of filtration or diffusion only, would necessarily be acid.

As the alkalinity of the blood increases the acidity of the urine decreases, until finally an alkaline urine results. The degree of the alkalinity of the blood, however, depends essentially upon the nature of the food and the secretion of the gastric juice, viz., the hydro-

[1] Brücke, Maly's Jahresber., 1887, vol. xvii. p. 189. Liebig, Annal. d. Chem. u. Pharmakol., 1844, vol. l. p. 61.

[2] Heintz, Jour. f. prakt. Chem., 1872, vol. vi. p. 274.

[3] F. Walters, Arch. f. exper. Path. u. Pharmakol., 1877, vol. vii. p. 148.

chloric acid. The ingestion of vegetable food, rich in salts of organic acids, which become oxidized in the body to the carbonates of the alkalies, will result in the passage of an alkaline urine, for the alkalies thus formed when absorbed into the blood are more than sufficient to neutralize completely all the acids present, and the elimination of neutral sodium phosphate alone takes place. In the case of animal food the reverse holds good. The alkaline carbonates here formed are not sufficient to neutralize the excess of acids, and diacid phosphate of sodium is hence eliminated in large quantity.[1]

An amphoteric urine results whenever the elimination of neutral and acid sodium phosphate is the same; such an occurrence is, therefore, more or less accidental.

As the alkalinity of the blood is increased during the secretion of the acid gastric juice, it may frequently happen, especially following the ingestion of a large amount of food, that an alkaline urine is voided. If this does not take place, the acidity of the urine is at least diminished, but increases again during the process of resorption of hydrochloric acid and peptones. The statement so generally found in text-books, that the urine secreted after a meal is alkaline, is not strictly correct; in a series of observations which I made in this direction an alkaline urine was observed in only 20 per cent. of the cases examined.[2]

It may thus be stated that an alkaline urine will result under physiological conditions whenever the alkaline salts present in the food are sufficient to neutralize all the acids formed, as occurs in the case of a vegetable diet, and, furthermore, whenever the period of gastric secretion is lengthened.

If an acid urine is allowed to stand exposed to the air for a certain length of time, its degree of acidity gradually diminishes, and the reaction finally becomes alkaline. At the same time the urine becomes cloudy and deposits a sediment, which consists of ammoniomagnesium phosphate, $MgNH_4PO_4+6H_2O$, neutral calcium phosphate, $Ca_3(PO_4)_2$, and still later contains ammonium urate, $C_5H_2(NH_4)_2N_4O_3$, in addition to the constituents of the primitive nubecula —*i. e.*, a few mucous corpuscles and pavement epithelial cells. The entire volume of urine, moreover, remains cloudy, owing to the presence of innumerable bacteria. The odor becomes extremely disagreeable, and distinctly "urinous." In short, "ammoniacal decomposition" has occurred. This has been shown to depend upon the action of certain bacteria, notably the Micrococcus ureæ and the Bacterium ureæ, which are present in the air.[3] These organisms cause decomposition of the urea found in every urine, with the formation of ammonium carbonate, according to the following equations:

[1] E. Salkowski u. J. Munk, Virchow's Archiv, 1877, vol. lxxvi. p. 500.

[2] Quincke, Zeit. f. klin. Med., vol. vii.

[3] W. Leube, "Ueber die ammoniakalische Harngährung," Virchow's Archiv, 1885, vol. c. p. 555.

$$CO(NH_2)_2 + 2H_2O = (NH_4)_2CO_3$$
$$(NH_4)_2CO_3 = 2NH_3 + H_2O + CO_2.$$

It is not the bacterium, however, which directly produces the result, but a bacterial product, and in this case an enzyme.

An alkaline urine, the alkalinity of which is not due to ammoniacal fermentation, however, but to other causes, as indicated above, may, of course, undergo the same change as an acid urine; but it is necessary to distinguish sharply between these two varieties of alkaline urines, as the recognition of the cause of the alkalinity is very often most important in diagnosis. The distinction is readily made by fastening a piece of sensitive red litmus-paper in the cork of the bottle containing the urine. If the alkalinity of the urine is due to the presence of ammonia, the litmus-paper will turn blue, but soon changes to red when exposed to the air; while a urine, the alkalinity of which is due to the presence of fixed alkalies, will turn red litmus-paper blue *only when immersed in the urine*, the change in color at the same time persisting.

As ammoniacal decomposition can also occur within the urinary passages, it is important, whenever an alkaline reaction due to the presence of ammonia is observed, to test the urine at once upon being voided, or, still better, to procure a portion with a catheter. Such urines are frequently seen in cases of cystitis the result of paralysis, urethral stricture, gonorrhœa, etc. In this connection it is interesting to note that whereas in old, neglected cases of cystitis an alkaline reaction is frequently observed, Brown has shown that in the great majority of cases of cystitis, both acute and chronic, and also in those of pyelitis and pyelonephritis. the urine is acid.[1]

An intensely acid reaction is observed in almost all concentrated urines, especially in fevers, in certain diseases of the stomach associated with a diminished or suspended secretion of hydrochloric acid, in gout, lithiasis, acute articular rheumatism, chronic Bright's disease, diabetes, leukæmia, scurvy, etc. Whenever a very acid urine is secreted for a considerable length of time, the possibility of renal irritation and the formation of concretions should be borne in mind.

An alkaline urine, the alkalinity of which is not owing to the presence of ammonia, but to a fixed alkali, is observed in certain cases of debility, especially in the various forms of anæmia, following the resorption of alkaline transudates, the transfusion of blood, frequent vomiting, a prolonged cold bath, etc. It may also be due to the ingestion of certain drugs, viz., salts of the organic acids and alkaline carbonates, the former being transformed into the latter, as has been mentioned. An increase in the degree of acidity may similarly take place after the ingestion of mineral acids.

Of interest is the observation of Pick[2] that in twenty-four to

[1] T. R. Brown, Johns Hopkins Hosp. Rep., 1901, vol. x. p. 11.
[2] F. Pick, "The Urine in Pneumonia," Münch. med. Woch., 1898, No. 17.

forty-eight hours after the crisis in pneumonia the urine shows a marked decrease in its acidity, becoming neutral or even alkaline. This phenomenon, which was observed in thirty-one out of thirty-eight cases, persists for a day or a day and a half, and then the acidity returns. In all likelihood the change is due to absorption of the large amounts of sodium which are present in the exudate.

An increase in the acidity of the urine upon standing has repeatedly been observed, and is probably due to the formation of new acids from pre-existing acid-yielding substances, such as certain carbohydrates, alcohol, etc., which have undergone fermentation. This phenomenon is frequently observed in diabetic patients.

A decrease in the acidity of normal urine upon standing, however, is the rule, owing to a gradual decomposition of sodium urate by the acid sodium phosphate, acid sodium urate, and, later on, uric acid resulting, which are thrown down as a sediment in consequence of the diminished acidity of the urine, and which, hence, no longer influence its reaction. This is shown in the equations:

(1) $NaH_2PO_4 + C_5H_2Na_2N_4O_3 = Na_2HPO_4 + C_5H_3NaN_4O_3$

(2) $NaH_2PO_4 + C_5H_3NaN_4O_3 = Na_2HPO_4 + C_5H_4N_4O_3$.

Determination of the Acidity of the Urine.—The old method of titrating a certain amount of urine with a decinormal solution of sodium hydrate has been abandoned and replaced by that of Freund. This is essentially based upon the observation that the acid reaction of the urine is referable exclusively to diacid phosphates.

Freund's Method.[1]—In 50 c.c. of urine the total amount of phosphoric acid is estimated as described on page 331. The result is termed *T*. In a second portion of 50 c.c. the monacid phosphates, *M*, are then precipitated with a normal solution of barium chloride—*i. e.*, one containing 122 grammes of the crystallized salt in 1000 c.c. of water—10 c.c. being added for every 100 mgrms. of the total amount of phosphoric acid found. After the addition of the barium the mixture is diluted to 100 c.c., filtered, and the phosphoric acid estimated in 50 c.c. of the filtrate. The result obtained is termed *D*. Owing to the fact that not only are the monophosphates precipitated on the addition of the barium chloride, but also a small amount of normal phosphates, and that a small amount of diacid phosphate is formed at the same time and passes into solution, an error is incurred. This, however, remains constant, and amounts to 3 per cent. in favor of the diacid phosphates.

As the total amount of phosphoric acid is subject to fairly wide variations, even in health, it is best to calculate the relative proportion of *T* to *D* for 100 c.c. of urine, and then to determine the absolute degree of acidity for the twenty-four hours. Figures are thus obtained which are directly comparable with one another.

[1] E. Freund, Centralbl. f. d. med. Wiss., 1892, p. 689.

Example.—Supposing that T amounted to 0.386 gramme for 100 c.c. of urine, and D to 0.338 gramme. Three per cent. of D would have to be deducted for reasons just given, making the true value of D 0.3279. The relative proportion of T to D would then be 84.9, as determined according to the equation

$$0.386 : 0.3279 :: 100 : x; \quad \text{and } x = 84.9.$$

Supposing, further, that the total amount of urine was 2000 c.c., the total acidity of the twenty-four hours would correspond to 1698, according to the equation $100 : 84.9 :: 2000 : x$; and $x = 1698$, and the total acidity per hour to $\frac{1698}{24}$, *i. e.*, 70.7.

The results obtained can also be expressed in terms of hydrochloric acid, 100 mgrms. of the diacid phosphates corresponding to 102.8 mgrms. of hydrochloric acid. This mode of indicating the total acidity of the urine would actually be the better.

If the urine should be alkaline and cloudy, the sediment is first dissolved by carefully adding a one-tenth or one-fourth normal solution of hydrochloric acid, the amount added being then deducted from the total acidity. Should negative values be found, these could be expressed in terms of sodium hydrate.[1]

With this method a complete revision of all the work previously done will be necessary. The results given above have reference only to the old method of titration with a one-tenth normal solution of sodium hydrate.

CHEMISTRY OF THE URINE.

General Chemical Composition of the Urine.—A general idea of the chemical composition of the urine and the quantitative variations of the individual components may be formed from the following table, which I have constructed from analyses made in my laboratory. The individuals from which the urines were obtained were adults, and their general mode of life, as regards diet, exercise, etc., was that of the average American city-dweller. In addition, the following substances may be encountered under pathological conditions: serum-albumin, serum-globulin, albumoses, mucin (nucleo-albumin), glucose, lactose, inosit, dextrin, biliary constituents, viz., bile-acids and bile-pigments, blood-pigments, melanin, leucin, tyrosin, oxybutyric acid, allantoin, fat, lecithin, cholesterin, acetone, alcohol, Baumstark's substance, urocaninic acid, cystin, hydrogen sulphide, and still others.

[1] The urine is carefully guarded against ammoniacal decomposition by the addition, to the first portion voided, of from 20 to 25 c.c. of a solution of 10 grammes of oil of peppermint to 100 c.c. of alcohol; or, a few cubic centimeters of chloroform are added, which answer the same purpose.

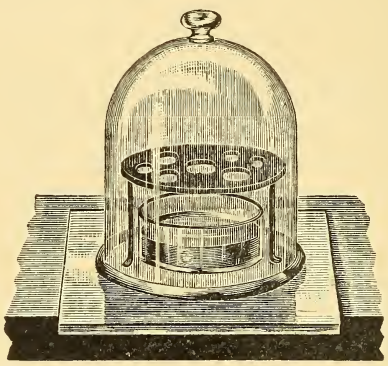
Analysis of Urine.

Water	1200 –1700	grammes.
Solids	60.0	"
Inorganic solids	25.0 –26.0	"
Sulphuric acid (H_2SO_4)	2.0 – 2.5	"
Phosphoric acid (P_2O_5)	2.5 – 3.5	"
Chlorine (NaCl)	10.0 –15.0	"
Potassium (K_2O)	3.3	"
Calcium (CaO)	0.2 – 0.4	"
Magnesium (MgO)	0.5	"
Ammonia (NH_3)	0.7	"
Fluorides, nitrates, etc.	0.2	"
Organic solids	20.0 –35.0	"
Urea	20.0 –30.0	"
Uric acid	0.2 – 1.0	"
Xanthin bases	1.0	"
Kreatinin	0.05– 0.08	"
Oxalic acid	0.05	"
Conjugate sulphates	0.12– 0.25	"
Hippuric acid	0.65– 0.7	"
Volatile fatty acid	0.05	"
Other organic solids	2.5	"

Quantitative Estimation of the Mineral Ash of the Urine.— In order to estimate the amount of mineral ash in the urine the following method may be employed:

Fifty c.c. of urine are evaporated to dryness in a weighed porcelain dish, at a temperature of 100° C., and then heated, while

Fig. 91.



Desiccator. (W. Simon.)

covered, over the free flame until gases cease to be evolved, care being taken not to heat too strongly in order to avoid sputtering. The residue is taken up with distilled boiling water, and, after standing, filtered through a Schleicher and Schüll's filter, the weight of the ash of which is known. The dish and the contents of the filter are well washed with hot water. Filtrate and washings are set aside and the dish and filter dried in the oven at 115° C. The

filter is now placed in the dish and slowly incinerated. So soon as the ash has turned white the filtrate and washings are placed in the same dish, evaporated at 100° C., and then carefully heated over the free flame. Upon cooling in the desiccator (Fig. 91) the dish with its contents is weighed, the difference between its present and previous weight indicating the quantity of ash contained in 50 c.c. of urine.

Precautions: 1. Care should be taken to allow the dish to become faintly red only for a moment, as some of the chlorine is otherwise volatilized. Some phosphoric acid may also escape, and too strong a heat, moreover, may cause the transformation of sulphates into sulphides, the organic material present acting as a reducing agent.

2. If the organic ash is not completely incinerated, it is best to allow the dish to cool and then to moisten the ash with a few drops of dilute sulphuric acid, when the heating is continued.

The Chlorides.

The chlorides which are excreted in the urine are derived from the food. As they are thus present in a much larger amount than all other inorganic salts combined, and in quantity more than sufficient to supply the needs of the body-economy, the relatively large amount of chlorides found in the urine under physiological conditions, as compared with the other inorganic constituents, is readily explained.

Of the alkalies in the urine, sodium in combination with chlorine exists in greatest amount, and for clinical purposes it is most convenient to calculate the total quantity of chlorides found in terms of sodium chloride; a small proportion also occurs combined with potassium, ammonium, calcium, and magnesium.

From 11 to 15 grammes of sodium chloride, representing the total quantity of chlorine, are normally eliminated in the twenty-four hours, the amount depending, of course, directly upon that contained in the food ingested. If the amount of nourishment is diminished, a decrease in the elimination of the chlorides is observed. If this is carried to the point of starvation, the chlorides disappear almost entirely from the urine, the traces remaining being derived from the body-fluids. The latter retain tenaciously a certain amount, which differs but slightly from that normally present. If at this stage food containing sodium chloride is again taken, a portion will be retained in the body until the original equilibrium is restored. A similar retention may be observed for a few days following the ingestion of large quantities of water, which causes an increased elimination of chlorides.

This tenacity on the part of the body in retaining sodium chloride

is strikingly seen when the potassium salt is substituted for the sodium salt; in this case the amount of the sodium in the serum of the blood will be found to vary very slightly.

It has also been shown that the excretion of sodium chloride can be increased very materially by the ingestion of potassium salts, notably the neutral potassium phosphate (K_2HPO_4). This is supposed to decompose the sodium chloride present in the serum, resulting in the formation of potassium chloride and neutral sodium phosphate, which are both eliminated as foreign material; a point is finally reached, however, when the sodium chloride ceases to be excreted.

This provision of the economy, in virtue of which an increase in the elimination of the salt is followed by its retention, and a previous retention by an increased elimination, is supposed to be referable to the albuminous metabolism taking place in the body. It may be stated, as a general rule, that any increase in the amount of circulating albumin will be followed by an increased elimination of chlorides, these having been previously retained by the albuminous bodies in consequence of the great affinity which exists between them. At the same time the elimination of the chlorides is influenced by the quantity of urine excreted, increasing and decreasing with its volume.

Pathologically the excretion of the chlorides may vary within wide limits, diminishing on the one hand to zero and increasing on the other to 50 grammes or more in the twenty-four hours. A marked diminution, which in some cases may go on to a total absence, was formerly thought to be pathognomonic of acute croupous pneumonia.[1] More modern investigations, however, have shown that such a condition occurs to a greater or less degree in most acute febrile diseases, such as scarlatina, roseola, variola, typhus and typhoid fevers, recurrens, and acute yellow atrophy.

The explanation of this phenomenon must be sought for, first, in a diminished ingestion of chlorides; secondly, in a retention of these in the blood, which probably is associated with an increase in the amount of the circulating albumin; thirdly, in a diminished renal secretion of water; fourthly, in a possible elimination of a portion of the chlorides through other channels, as in cases of severe diarrhœa, the formation of serous exudates, etc.[2] Intermittent fever appears to be an exception to this rule; usually it is true the chlorides are diminished, but not to the extent seen in the other diseases mentioned. They have, moreover, been found to increase during and sometimes immediately after a paroxysm, this increase being, of course, followed by a corresponding diminution.

The chlorides are diminished in all acute and chronic renal dis-

[1] Rettenbacher, Wien. med. Zeit., 1850, p. 373. Heller, Heller's Archiv, 1844, vol. i. p. 23. [2] Salkowski u. Leube, Lehre vom Harn, 1882, p. 174.

eases associated with albuminuria, owing to some extent at least to a diminished secretion of water.[1]

In all cases of carcinoma of the stomach, and in chronic hypersecretion associated with dilatation, a decrease is also observed, which in certain cases of hypersecretion and hyperacidity, the result of gastric ulcer, may go on to a total absence.[2]

In anæmic conditions the chlorides are likewise diminished, as also in rickets. In melancholia and idiocy a striking decrease is observed; in dementia, chorea, and pseudohypertrophic paralysis this is less marked. A total absence has been noted in pemphigus foliaceus, and a considerable diminution in the beginning of impetigo, as also in chronic lead poisoning.

The chlorides are found in increased amount, on the other hand, in all conditions in which retention has previously occurred, chief among these being the acute febrile diseases and cases in which a resorption of exudates and transudates, associated with an increased diuresis, is taking place. A marked increase has also been noted in some cases of diabetes insipidus, in which 29 grammes have been eliminated in the twenty-four hours.[3] A similar increase may occur in prurigo, in which, in one instance, 29.6 grammes were passed in twenty-four hours.[4] In cases of general paresis, during the first stage, an increased elimination goes hand in hand with an increased ingestion of food. In epilepsy the polyuria following the attacks is associated with an increase in the chlorides.

Of drugs, certain diuretics, and some of the potassium salts, as has been mentioned, produce an increase: the chlorine contained in chloroform, whether administered internally or as an anæsthetic, is in part excreted in the form of a chloride. Salicylic acid, on the other hand, is said to cause a temporary diminution.

It is of practical importance to note that in acute febrile diseases the diminution in the chlorides appears to vary with the intensity of the disease, a decrease to 0.05 gramme pro die justifying the conclusion that the case under observation is of extreme gravity. It may at times also indicate the previous occurrence of severe diarrhœa or the formation of exudates of considerable extent. A continued increase, on the other hand, should lead to the conclusion that the patient's condition is improving.

The elimination of the chlorides also furnishes a fair index to the digestive powers of the patient. This rule also holds good for most chronic diseases. All other causes which might lead to an increase or decrease being eliminated, an excretion of from 10 to 15 grammes indicates a fair condition of the appetite and a normal digestive power, a decrease being associated with the reverse.

[1] Röhmann, Zeit. f. klin. Med., 1886, vol. i. p. 513.
[2] Gluzinski, Berlin. med. Woch., 1887, vol. xxiv. p. 983.
[3] Oppenheim, Zeit. f. klin. Med., vol. vi.
[4] v. Brueff, Wien. med. Woch., 1871, p. 552.

An increased elimination of chlorides occurring in cases of œdema, and associated with the existence of serous exudates, is always of good prognostic omen, pointing to a resorption of the fluid.

A continued elimination of more than 15 to 20 grammes, all other causes being excluded, may be considered as pathognomonic of diabetes insipidus.

Test for Chlorides in the Urine.—The recognition of the chlorides in the urine is based upon the fact that the addition of a solution of silver nitrate causes their precipitation, the reaction taking place according to the equation

$$AgNO_3 + NaCl = AgCl + NaNO_3.$$

The silver chloride thus formed is insoluble in nitric acid.

The test is made in the following manner: after having removed any albumin that may be present, according to methods given elsewhere (see Albumin), a few cubic centimeters of urine are acidified in a test-tube with about 10 drops of pure nitric acid, and treated with a few cubic centimeters of silver nitrate solution (1 : 20). The occurrence of a white precipitate indicates the presence of chlorides. An idea may be formed at the same time of the quantity present; the occurrence of a heavy, caseous precipitate points to a large amount.

Quantitative Estimation of the Chlorides by the Method of Salkowski-Volhard.[1]—When a solution of silver nitrate acidified with nitric acid is treated with a solution of potassium sulphocyanide or ammonium sulphocyanide, in the presence of a ferric salt, the potassium sulphocyanide first causes the precipitation of white silver sulphocyanide, which, like silver chloride, is insoluble in nitric acid:

$$AgNO_3 + KSCN = AgSCN + KNO_3.$$

As soon as every trace of silver is precipitated, it combines with the ferric salt to form ferric sulphocyanide, which is of a blood-red color:

$$6KSCN + Fe_2(SO_4)_3 = Fe_2(SCN)_6 + 3K_2SO_4.$$

If the potassium sulphocyanide solution is of known strength, it is possible to estimate accurately the amount of silver present in the solution, the ferric salt serving as an indicator of the end of the reaction between the silver and the potassium sulphocyanide.

Application to the urine: to urine which has been acidified with nitric acid an excess of a silver solution of known strength is added, and the silver not used in the precipitation of the chlorides then estimated as indicated above. The difference between the quantity thus found and the total amount used will be that consumed in the pre-

[1] E. Salkowski, Zeit. f. physiol. Chem., vol. i. p. 16, and vol. ii. p. 397.

cipitation of the chlorides, from which, knowing the strength of the silver solution, its equivalent in terms of sodium chloride is readily determined.

Reagents required:

1. A solution of silver nitrate of such strength that each cubic centimeter shall correspond to 0.01 gramme of sodium chloride.

2. A solution of potassium sulphocyanide of such strength that 25 c.c. shall correspond to 10 c.c. of the silver nitrate solution.

3. A solution of a ferric salt, such as ammonio-ferric alum, saturated at ordinary temperature.

4. Nitric acid (specific gravity 1.2).

Preparation of these solutions:

1. As pointed out, the silver nitrate solution is made of such strength that each cubic centimeter shall correspond to 0.01 gramme of sodium chloride; in other words, a standard solution is employed.

The silver nitrate must be pure, and it is best to use the crystallized salt, and not the sticks wrapped in paper, which always contain reduced silver. In order to test the purity of the salt, about 1 gramme is dissolved in distilled water, heated to the boiling-point, the silver precipitated by dilute hydrochloric acid and filtered off. When evaporated in a platinum crucible the filtrate should leave either no residue at all or only a very faint one; otherwise it is necessary to recrystallize the salt until the desired degree of purity is reached.

The determination of the quantity to be dissolved in 1000 c.c. of water is based upon the fact that 1 molecule of silver nitrate (molecular weight 170) combines with 1 molecule of sodium chloride (molecular weight 58.5) to form silver chloride and sodium nitrate. As the solution of silver nitrate shall be of such strength that 1 c.c. corresponds to 0.01 gramme of sodium chloride, or 1000 c.c. to 10 grammes, the quantity to be dissolved in 1000 c.c. is found according to the following equation:

$$58.5 : 170 : : 10 : x,\ 58.5\,x = 1700,\ x = 29.059.$$

Theoretically, then, this quantity should be dissolved in 1000 c.c. of water. It is better, however, to dissolve it in a quantity somewhat less than 1000 c.c., such as 900 or 950 c.c., as the silver salt contains water of crystallization and the weighed-off quantity would not represent the exact amount required, but less, the correcting of a solution which is too strong being a much simpler matter than that of a solution which is too weak.

To make this correction, or, in other words, to bring the solution to its proper strength, 0.15 gramme of sodium chloride which has previously been dried carefully by heating in a platinum crucible, is accurately weighed off, dissolved in a little distilled water, and further diluted to about 100 c.c. To this solution a few drops of a solution

of potassium chromate are added, when the mixture is titrated with the silver solution.

The silver nitrate will first precipitate the sodium chloride, and then combine with the potassium chromate, forming red silver chromate, according to the equation

$$2AgNO_3 + K_2CrO_4 = Ag_2CrO_4 + 2KNO_3.$$

The slightest orange tinge remaining after stirring indicates the end of the reaction. Were the solution of the silver nitrate of the proper strength, exactly 15 c.c. should have been used, as each cubic centimeter shall represent 0.01 gramme of sodium chloride. As a matter of fact, less will in all probability be needed, the solution having been purposely made too strong. Its correction then becomes a simple matter, as it is merely necessary to determine the degree of dilution required.

Supposing that 29.059 grammes of silver nitrate were dissolved in 900 c.c. of water, and that 14.5 c.c. instead of 15 c.c. had been required to precipitate the 0.15 gramme of sodium chloride, it is evident that each 14.5 c.c. of the remaining solution must be diluted with 0.5 c.c. of water. It is, hence, only necessary to divide the number of cubic centimeters of the silver nitrate solution remaining by 14.5; the result multiplied by 0.5 represents the amount of water which must be added in order to bring the solution to the required strength. Hence the rule for the correction of a solution which has been found too strong:

$$C = \frac{N.\ d}{n},$$

in which C represents the number of cubic centimeters of water which must be added to the solution remaining; N the total number of cubic centimeters remaining after titration; n the number of cubic centimeters consumed in one titration; and d the difference between the number of cubic centimeters theoretically required and that actually used in one titration.

In the example given the equation would then read:

$$C = \frac{936.5 \times 0.5}{14.5} = 32.29.$$

32.29 c.c. of distilled water are added to the remaining 936.5 c.c., when the strength of the solution is tested by a second titration. If the solution is found too weak, it is best to make it too strong, and then to correct as described.

2. Preparation of the potassium sulphocyanide solution: from the equation $AgNO_3 + KSCN = AgSCN + KNO_3$, it is seen that 1 molecule of silver nitrate (molecular weight 170) combines with 1 molecule of potassium sulphocyanide (molecular weight 97). The

quantity of the latter to be dissolved in 1000 c.c. of water is then found from the following equation:

$$170 : 97 : : 11.6236 : x; \quad 170\,x = 11.6236 \times 97; \quad x = 6.6.$$

As potassium sulphocyanide is extremely hygroscopic, a solution is made which is too strong, by dissolving about 10 grammes of the salt in 900 c.c. of distilled water. In order to bring this solution to its proper strength, 10 c.c. of the silver solution are diluted to 100 c.c.; 4 c.c. of nitric acid (specific gravity 1.2) and 5 c.c. of the ammonio-ferric alum solution are added, when the mixture is titrated with the potassium sulphocyanide solution; the end-reaction is recognized by the production of a slightly reddish color, which persists on stirring. The sulphocyanide solution having been purposely made too strong, it will be found that less than 25 c.c. are needed to precipitate all the silver present. The quantity of water necessary for dilution is ascertained, as above, according to the formula

$$C = \frac{N.d}{n}.$$

3. The solution of ammonio-ferric alum is a solution saturated at ordinary temperatures, care being taken to insure the absence of chlorides in the salt, which may be effected, if necessary, by recrystallization.

Method as Applied to the Urine.—Ten c.c. of urine are placed in a small stoppered flask bearing a 100 c.c. mark, diluted with 50 c.c. of distilled water, and acidified with 4 c.c. of nitric acid. From a burette 15 c.c. of the standard solution of silver nitrate are added. The mixture is thoroughly agitated and diluted with distilled water to the 100 c.c. mark. The silver chloride formed is filtered off through a *dry* folded filter into a *dry* graduate; 80 c.c. of the filtrate are placed in a beaker, and, after the addition of 5 c.c. of the ammonio-ferric alum solution, titrated with the sulphocyanide solution until the end-reaction—*i. e.*, a slightly reddish tinge—is seen. If necessary, two such titrations should be made, the sulphocyanide solution being added 1 c.c. at a time in the first, while in the second the total number of cubic centimeters needed to bring about the end-reaction, less 1 c.c., are added at once, and then 0.1 c.c. at a time.

The amount of chlorides present in the urine is calculated as follows:

Example.—Total quantity of urine 600 c.c.; 6.5 c.c. of the sulphocyanide solution were required to bring about the end-reaction in 80 c.c. of the filtrate; this would correspond to 8.125 c.c. for the total 100 c.c. of filtrate, representing 10 c.c. of urine, as is seen from the equation

$$n : 80 : : x : 100; \quad 80\,x = 100\,n; \quad x = \frac{100\,n}{80} = \frac{5\,n}{4},$$

in which x represents the number of cubic centimeters corresponding to 100 c.c. of the filtrate, and n the number of cubic centimeters actually used.

These 8.125 c.c. were used in precipitating the silver nitrate not decomposed by the chlorides. As 25 c.c. of the sulphocyanide solution correspond to 10 c.c. of the silver solution, the excess of silver solution in cubic centimeters is found from the equation

$$25 : 10 :: N : x; \quad 25x = 10N; \quad x = \frac{10N}{25} = \frac{2N}{5},$$

in which x represents the excess of the silver solution in cubic centimeters, and N that of the sulphocyanide solution as found according to the equation above, x in this case being 3.25 c.c.

The difference between the total amount of silver solution employed (*i. e.*, 15 c.c.) and the excess (*i. e.*, 3.25 c.c.) indicates the number of cubic centimeters necessary for the precipitation of the chlorides in 10 c.c. of urine. In the case under consideration 11.75 c.c. were employed. As 1 c.c. of the silver solution represents 0.01 gramme of sodium chloride, there must have been present in the 10 c.c. of urine 0.1175 gramme; in 100 c.c., hence, 1.175 grammes, and in the total amount—*i. e.*, 600 c.c. of urine—7.05 grammes.

From these considerations the following short rule results: instead of first multiplying the number of cubic centimeters of the potassium sulphocyanide solution corresponding to 80 c.c. of the filtrate, by $\frac{5}{4}$, and the result by $\frac{2}{5}$, in order to find the number of cubic centimeters of the potassium sulphocyanide solution representing the excess of silver nitrate in 100 c.c. of the filtrate, and then deducting the result from 15, it is simpler to multiply by $\frac{1}{2}$ directly and deduct the result from 15, the number of grammes of sodium chloride contained in 1000 c.c. of urine being thus found. This figure is then corrected for the total amount of urine.

The method described may be employed in the presence of albumins, albumoses, and sugar; the urine, however, must be fresh, so as to insure the absence of nitrous acid.

Direct Method.[1]—If absolute accuracy is not required, the following method may be employed:

Ten c.c. of urine are diluted with distilled water to 100 c.c. and treated with a few drops of a solution of potassium chromate. This mixture is titrated with a one-tenth normal solution of silver nitrate until the end-reaction is reached—*i. e.*, a faint orange tinge—which no longer disappears on stirring. The number of cubic centimeters used multiplied by 0.01 will indicate the amount of chlorides present in 10 c.c. of urine.

As uric acid, the xanthin-bases, hyposulphites, sulphocyanides,

[1] F. Mohr, Lehrbuch d. Titrirmethode, 1856, ii. p. 13.

and pigments are also precipitated by the silver nitrate, the end-reaction is delayed; moreover, unless the urine is very pale, its recognition may be difficult, and the error thus caused considerable. This is especially true of febrile urines which contain only a small amount of chlorides.

Should iodides or bromides have been taken, these must first be removed, as silver iodide and bromide, which are insoluble in nitric acid, would give too high a value.

To this end, the following method, which is also a very accurate one, should be employed, its only disadvantage being the amount of time required.

Estimation of the Chlorides after Incineration (according to Neubauer and Salkowski).[1]—The principle of this method is the destruction of all organic material and the subsequent estimation of the chlorides contained in the mineral ash by one of the methods described. Ten c.c. of urine are evaporated to dryness in a platinum crucible at a temperature slightly below 100° C., after the addition of a little pure dried sodium carbonate and from 3 to 5 grammes of potassium nitrate. The addition of the sodium carbonate insures the conversion of any ammonium chloride which may be present into sodium chloride; the potassium nitrate acts merely as an oxidizing agent. The residue is now carefully heated at a moderate temperature, allowed to cool, dissolved in distilled water, and accurately neutralized with very dilute nitric acid. In this solution the chlorides are estimated most conveniently according to the second method.

Should iodides or bromides be present, the aqueous solution just referred to is acidified with sulphuric acid, and the iodine and bromine thereby liberated extracted with carbon disulphide. As complete removal of these bodies is, however, only possible in the presence of a nitrite, it is better not to rely upon the presence of any that may have been formed during the process of incineration, but to add a few drops of a solution of potassium nitrite. After extraction the nitrous acid is decomposed by the addition of a little urea. The solution is then neutralized with sodium carbonate; should it be alkaline, dilute acetic acid is added until neutral. In this solution the chlorides are most conveniently estimated according to the second method.

Albumin and sugar, if present, should be removed before the addition of the sodium carbonate and potassium nitrate, so as to obviate losses from sputtering, which otherwise would occur. Nitrous acid must also be removed for reasons given above.

The Phosphates.

The phosphates occurring in the urine are sodium, potassium, calcium, and magnesium salts of the tribasic acid H_3PO_4. The most

[1] E. Salkowski, Pflüger's Archiv, vol. vi. p. 214.

important of these, as was pointed out in the chapter on Reaction, is the diacid sodium phosphate NaH_2PO_4, to which the acidity of the urine is due. It is owing to the presence of this salt in the urine that the calcium phosphate is held in solution; the fact, at least, that calcium and magnesium phosphates are thrown down when the urine is neutralized, would point to this conclusion.

The composition of the phosphates is liable to considerable variation, depending upon the degree of acidity of the urine. As would be expected, diacid sodium phosphate and diacid calcium phosphate are present in an acid urine; in an amphoteric urine, in addition to these there are found disodium phosphate, monocalcium phosphate, and monomagnesium phosphate, while in an alkaline urine trisodic phosphate, neutral calcium phosphate, and neutral magnesium phosphate may be present.

The alkaline phosphates normally exceed the earthy phosphates by one-third, and sodium is combined with far the greater amount of phosphoric acid, the potassium salt normally occurring in only very small amounts.

In addition to the mineral phosphates, phosphoric acid is excreted also in combination with glycerin as glycerin-phosphoric acid, which need not, however, be considered in a quantitative estimation, as it is present only in traces.[1]

As in the case of the chlorides, the greater portion of the phosphates is derived from the food, while only a small portion is referable to the phosphorus built up in the proteid molecule, be this in the form of a muscle-cell, a nerve-cell, a red blood-corpuscle, or bone. But just as the percentage of sulphur varies in the different tissues, so also does that of phosphorus vary; nerve-tissue, for example, which is very rich in lecithin and nucleins, yields relatively more phosphorus than muscle-tissue.

Not all the phosphoric acid ingested, however, is excreted in the urine, as one-third to one-fourth of the total quantity is eliminated in the feces.

The quantity of phosphoric acid excreted, which normally varies between 2.5 and 3 grammes, is thus largely dependent upon the amount ingested, increasing with an animal and decreasing with a vegetable diet.[2] During starvation a considerable increase is likewise observed, referable, no doubt, to an increased destruction of bony tissue, which is very rich in the phosphates of the alkaline earths. In accordance with this view, increased amounts of calcium and magnesium are also seen during starvation. The relation between the excretion of phosphoric acid and nitrogen, normally 1 : 7, changes, moreover, in such a manner that both the absolute and the relative amount of phosphoric acid, as compared with the nitrogen, increases;

[1] Lépine et Eymonnet, Compt. rend. de la Soc. de Biol., 1882.
[2] Zülzer, Virchow's Archiv, vol. lxvi. p. 223.

this leads to the conclusion that in addition to the muscles some other tissue rich in phosphorus and relatively poor in N must suffer during the process, and the only one which could enter into consideration is bone.[1] If at this time food containing phosphorus is again given, a retention will take place, so that the general rule stated in the chapter on Chlorides, that increased elimination is followed by a certain degree of retention, and that a previous retention is followed by an increased elimination, seems to hold good for all the mineral acids found in the urine (see also the chapter on Sulphates).

An increased elimination is caused also by the ingestion of large quantities of water, which is followed by a certain degree of retention.

Observations on the phosphatic excretion during muscular exercise have not given uniform results.[2] Mental exercise appears to cause a diminished excretion of the alkaline phosphates and an increased elimination of the earthy phosphates.[3] The latter also takes place during sleep.

In disease the total amount of phosphates may either be increased or diminished.

A *diminished* elimination is observed in most cases of acute febrile disease, such as pneumonia, typhoid fever, typhus fever, recurrens, during a paroxysm of intermittent fever, etc. The degree of diminution is usually proportionate to the severity of the disease, reaching its lowest figure as death approaches. Such a state of affairs may, at first sight, appear paradoxical in view of what has been said above of the effects of tissue-destruction upon the elimination of phosphates. It is necessary, however, to distinguish sharply between an increased production and an increased elimination ; in all probability a retention occurs analogous to that of the chlorides, which may be observed under the same conditions. It has been supposed that the phosphates set free during the process of tissue-destruction are utilized in the building up of new leucocytes, and an increase in these is actually noted in some of the diseases mentioned. A diminished excretion of phosphates is, however, not always observed, and an increased elimination may occur in certain cases. In fatal cases this condition may persist even until the time of death. It is very difficult to give a satisfactory explanation of this fact at the present time. The phenomenon, in typhoid fever at least, appears to be connected with the intensity of the nervous manifestations, and Robin concludes that here an increased elimination during the fastigium is an unfavorable omen, while an increase during defervescence warrants a favorable prognosis. A similar decrease in the phosphates has also been observed in pulmonary phthisis associated with high fever.[4]

[1] Zülzer, loc. cit.
[2] Fleischer u. Penzoldt, Virchow's Archiv, vol. lxxxvii. p. 210.
[3] Mairet, Compt. rend. de la Soc. de Biol., 1884.
[4] Edlefsen, Schmidt's Jahresber., vol. cxcvi. p. 59.

Very interesting and important is the diminished excretion of the phosphates associated with acute and, to some extent also, with chronic nephritis, amyloid degeneration of the kidneys, and the anæmias, in which an actual insufficiency on the part of the kidneys in the elimination of these salts appears to exist.[1]

A diminished or, at least, no increased excretion is seen in certain diseases of the bones, such as osteomalacia, although an increase in the *earthy* phosphates has been noted. This may depend either upon a retention or an elimination through other channels. The *earthy* phosphates especially are found in greatly diminished amount, or may even be absent altogether in certain cases of nephritis. A similar condition is observed in acute and chronic rheumatism.

The data regarding the phosphatic elimination in nervous and mental diseases are, on the whole, scanty and by no means uniform.

During attacks of hysteria major, in contradistinction to epilepsy, in which an increased elimination takes place, the phosphates are diminished, the degree of diminution being generally proportionate to the intensity of the attack, increasing again together with the other urinary constituents with the subsequent increase in the diuresis.[2]

In chronic lead poisoning a diminution to one-third of the normal quantity may occur. Very low figures have been noted in Addison's disease, in acute yellow atrophy (in which even a total absence may occur), and in certain cases of hepatic cirrhosis. In gout the phosphoric acid curve follows that of the uric acid quite closely, decreasing before the onset of the acute symptoms and then rising and reaching its maximum about the third day (see Uric Acid).[3]

An *increased* elimination of phosphates, on the other hand, amounting in some cases to 7 or even to 9 grammes in the twenty-four hours, has been described by Teissier, of Lyon, under the name of *phosphatic diabetes*, the patient presenting various symptoms commonly seen in diabetes mellitus; sugar, however, is usually absent. Whether or not phosphatic diabetes is a disease *sui generis* is not certain.[4]

In true diabetes mellitus a curious relation has been found to exist between the elimination of sugar and of phosphates, the quantity of the latter rising and falling in an inverse ratio to the amount of sugar. In diabetes insipidus a slight increase is at times found.

Corresponding to the phosphatic retention observed in acute febrile diseases an increased elimination is noted during convalescence. An increase occurs in the course of cerebrospinal meningitis.

In a case of pseudoleukæmia an increase of 7 grammes has been noted, while the number of red corpuscles fell from 2,200,000 to 800,000 in four days. To judge from the very careful observations

[1] Fleischer, Deutsch. Arch. f. klin. Med., vol. xxix. p. 129.
[2] De la Tourette and Cathelineau, Centralbl. f. d. med. Wiss., 1889, vol. xlviii. p. 872.
[3] T. B. Futcher, Jour. Am. Med. Assoc., 1902, vol. xxxix. p. 1046.
[4] G. Rankin, "Phosphatic Diabetes," Lancet, March 24, 1900. Teissier, Thèse, Paris, 1876.

made, there could be no doubt that the high degree of phosphaturia, which was limited to the alkaline phosphates, was referable to this latter source. In leukæmia also an increase to 7 grammes has been observed on the day preceding death; commonly, however, the increase is but slight in this disease.[1]

While it is apparent that important conclusions cannot be drawn, on the whole, from a knowledge of the absolute phosphatic elimination, unless it be from a study of the relation existing between the excretion of the alkaline and earthy phosphates, a study of the *relative phosphatic excretion* seems to promise more valuable results. According to Zülzer,[2] a definite amount of the phosphates and of the urinary nitrogen is referable to the destruction of albuminous material, so that the relation between the phosphoric acid and the nitrogen must be constant. Another portion, however, is derived from lecithin, one of the most important constituents of nerve-tissue, which contains more phosphorus than the albuminous molecule. Whenever, then, the lecithin-containing tissues are more involved in the general metabolism than under normal conditions the relation will no longer be a stable one. This relation which exists between the elimination of nitrogen and phosphoric acid has been termed the *relative value* of phosphoric acid.

The relative value of phosphoric acid in the urine has been calculated as varying from 17 to 20, that of the blood being 3, of muscle-tissue 12.1, of brain 44, of bone 426 to 430. This value supposes the absolute value to vary between 2 and 3 grammes pro die. It is found according to the following equation:

$$N : P_2O_5 :: 100 : x; \text{ and } x = \frac{100 \cdot P_2O_5}{N},$$

in which N indicates the amount of nitrogen actually observed, P_2O_5 the amount of phosphoric acid in the same specimen of urine, and x the amount of P_2O_5 corresponding to 100 grammes of N. By observing this relative value a much better idea may be formed of the metabolic processes taking place in the body in disease than from a mere expression of the absolute phosphatic value.

In acute febrile diseases the relative as well as the absolute diminution of the phosphates has been ascribed to a retention, they being possibly utilized in the building up of white blood-corpuscles. In the course of these diseases oscillations in the relative value are frequently observed; during convalescence the relative as well as the absolute value again rises.

In accordance with these considerations a diminished relative excretion of phosphoric acid should be expected in all cases associated with a notable elimination of leucocytes through other channels, as in pneumonia, for example, or a storing away of the same, as in

[1] Fleischer u. Penzoldt, loc. cit. [2] Loc. cit.

cases of empyema. The facts observed are in accord with this view.

A relative decrease has further been noted in the various forms of anæmia, conditions of cerebral excitation, and especially preceding an attack of epilepsy. In progressive paralysis following syphilis the relative value, at first low, rises greatly after the administration of potassium iodide, while the excretion of the earthy phosphates is lessened. In chronic cerebral affections, delirium tremens, and acute hydrocephalus a relative decrease has been noted. In mania, during the period of excitement, both the alkaline and the earthy phosphates are found increased, while during the stage of depression, as also in melancholia, the alkaline phosphates are diminished and the earthy phosphates increased. On the other hand, an increase in the relative value has been noted in apoplexy (amounting to 34.3 in one case, two days after an attack), brain tumors, tabes, arthritis deformans (30), pernicious anæmia (23.8–58), etc.[1]

Of drugs, bromides appear to diminish the absolute amount of phosphoric acid. Cocain and quinin cause a decrease, and salicylic acid an increase. A relative decrease is produced by the cerebral excitants, such as strychnin, small doses of alcohol, phosphorus, valerian, cold baths, salt-water baths, etc. An opposite effect is produced by the cerebral depressants, such as chloroform, morphin, chloral, large doses of alcohol, potassium bromide, mineral and vegetable acids, prolonged cold baths, Turkish baths, low temperature, etc.

Tests for the Phosphates in the Urine.—The test for the detection of the phosphates occurring in the urine depends upon the precipitation of phosphoric acid by means of ferric chloride as ferric phosphate, which is insoluble in cold acetic acid:

$$2NaH_2PO_4 + Fe_2Cl_6 = Fe_2(PO_4)_2 + 2NaCl + 4HCl,$$

or

$$2Na_2HPO_4 + Fe_2Cl_6 = Fe_2(PO_4)_2 + 4NaCl + 2HCl.$$

The same result may be accomplished by the addition of a solution of uranyl nitrate; this gives rise to the formation of uranyl phosphate, which is also insoluble in acetic acid:

$$Na_2HPO_4 + 2UO.NO_3 = 2NaNO_3 + (UO)_2HPO_4,$$

or

$$Na_2HPO_4 + UO.NO_3 = NaNO + UO.H_2PO_4.$$

Test.—A few cubic centimeters of urine are acidified with a few drops of acetic acid, and treated with a few drops of a solution of ferric chloride (1 part of the officinal solution to 10 parts of water), when the occurrence of a yellowish-white precipitate will indicate the presence of phosphates.

[1] Zülzer u. Edlefsen, loc. cit.

If a solution containing an acid phosphate of the alkalies is treated with an alkaline hydrate, the diacid alkaline phosphate is transformed into the monacid salt, according to the equation

$$NaH_2PO_4 + NH_4OH = NaNH_4HPO_4 + H_2O.$$

This is further changed into the normal salt, as represented:

$$3NaNH_4HPO_4 + NH_4OH = Na_3PO_4 + (NH_4)_3(PO_4)_2 + H_2O.$$

As the monacid and neutral salts are both readily soluble, the solution remains clear. If at the same time, as in the urine, a soluble diacid phosphate of the alkaline earths is present, this likewise is transformed into the monacid and finally into the neutral salt; the latter, however, being insoluble, is thrown down:

$$(1)\ Ca(H_2PO_4)_2 + 4NH_4OH = Ca(NH_4)_2(PO_4)_2 + 4H_2O.$$
$$(2)\ 3Ca(NH_4)_2(PO_4)_2 = Ca_3(PO_4)_2 + 2(NH_4)_3PO_4.$$

Test for the Earthy Phosphates.—Ten c.c. of urine are rendered alkaline with ammonia, when the occurrence of a flocculent precipitate will indicate their presence.

Test for the Alkaline Phosphates.—After having removed the earthy phosphates from 10 c.c. of urine, as just described, the clear filtrate is acidified with acetic acid and tested with ferric chloride or uranyl nitrate, as shown above.

The alkaline phosphates may also be detected by treating the ammoniacal filtrate with a few drops of *magnesia mixture* (1 part of crystallized magnesium sulphate, 2 parts of ammonium chloride, 4 parts of ammonium hydrate, and 8 parts of distilled water), when ammonio-magnesium phosphate, which is almost insoluble in ammonium hydrate, will be thrown down. The reaction takes place between the monacid or neutral sodium phosphate and the magnesium sulphate according to the equation

$$Na_2HPO_4 + MgSO_4 + NH_4OH + NH_4Cl = MgNH_4PO_4 + NH_4Cl + Na_2SO_4 + H_2O.$$

Quantitative Estimation of the Total Amount of Phosphates.—*Principle.*—When a solution of disodium phosphate acidified with acetic acid is treated with a solution of uranyl nitrate or uranyl acetate, a dirty-looking, white precipitate of uranyl phosphate is thrown down, which is formed according to the equation given above. It is apparent that the quantity of phosphoric acid can be estimated accurately, if the solution of uranyl nitrate or acetate is of known strength.

Solutions required:

1. A solution of uranium nitrate of such strength that 20 c.c. shall correspond to 0.1 gramme of P_2O_5.

2. A solution containing sodium acetate and acetic acid.
3. Tincture of cochineal.

Preparation of these solutions:

1. From the equation

$$2UO.NO_3 + Na_2HPO_4 = (UO)_2HPO_4 + 2NaNO_3$$

it is apparent that 2 molecules of uranium nitrate combine with 1 molecule of disodium phosphate to form uranium phosphate and sodium nitrate. The molecular weight of uranium nitrate being 318 and that of disodium phosphate 142, it is seen that 636 parts by weight of the former combine with 142 parts by weight of the latter.

As 20 c.c. of the solution of uranium nitrate shall correspond to 0.1 gramme of P_2O_5, 1000 c.c. must be equivalent to 5 grammes of P_2O_5. In 142 parts by weight of disodium phosphate there would be present 71 grammes of P_2O_5, equivalent to 636 parts by weight of uranium nitrate. The quantity of the latter, then, to be dissolved in 1000 c.c. of water will be found from the equation: 636 : 71 : : x : 5; and $x = 44.78$.

44.78 grammes of uranium nitrate are weighed off and dissolved in about 900 c.c. of water, the solution being purposely made too strong for reasons pointed out in the chapter on Chlorides. In order to bring this solution to its proper strength it is necessary to titrate with the uranium solution a solution of disodium phosphate of such strength that each 50 c.c. shall contain 0.1 gramme of P_2O_5, or 1000 c.c. 2 grammes. The molecular weight of $Na_2HPO_4 + 12H_2O$ being 358, this amount of disodium phosphate in grammes is equivalent to 142 grammes of P_2O_5; the quantity of P_2O_5 corresponding to 2 grammes, in terms of $Na_2HPO_4 + 12H_2O$, is found from the equation: 358 : 142 : : x : 2; and $x = 5.042$. This amount of pure, dry, and non-deliquescent Na_2HPO_4 is dissolved in 1000 c.c. of distilled water. If non-deliquescent disodium phosphate is not at hand, about 6 or 7 grammes of the salt are dissolved in 1000 c.c. of distilled water; of this solution 50 c.c. are evaporated in a weighed platinum dish, and the residue gently heated, the disodium phosphate being thereby transformed into sodium pyrophosphate, $Na_4P_2O_7$, according to the equation

$$2Na_2HPO_4 = Na_4P_2O_7 + H_2O.$$

The molecular weight of $Na_4P_2O_7$ being 266, this corresponds to 142 grammes of P_2O_5. If the solution is of the correct strength —*i. e.*, containing 0.1 gramme of P_2O_5 in 50 c.c. of water—the residue should weigh 0.1873 gramme, as is seen from the equation: 132 : 266 : : 0.1 : x; and $x = 0.1873$. Supposing, however, that the residue weighs 0.1921 gramme, it is manifest that the solution is

too strong, and must be diluted, the degree of dilution being ascertained according to the equation : 0.1873 : 1000 : : 0.1921 : x ; and $x = 1025$; *i. e.*, 1000 c.c. of the solution must be diluted to 1025 c.c. to make it of the proper strength.

In the case given, 50 c.c. were used ; the 950 c.c. are then diluted with the amount of water found from the equation : 1000 : 1025 : : 950 : x ; and $x = 973.75$. Having thus obtained a solution of disodium phosphate of such strength that each 50 c.c. shall contain 0.1 gramme of P_2O_5, this is titrated with the uranium solution, which has been made too strong, in order to determine the amount of water that must be added to the latter. To this end, a burette is filled with the uranium solution ; 50 c.c. of the disodium phosphate solution are treated with a few drops of the tincture of cochineal and 5 c.c. of the acetic acid mixture (see below). This mixture is heated in a beaker, and as soon as the boiling-point has been reached titrated with the uranium solution until a trace of a greenish color is noticed in the precipitate which does not disappear on stirring. This point having been accurately determined by means of a second titration, the number of cubic centimeters of distilled water with which the remaining solution must be diluted is determined according to the formula : $C = \frac{N.\,d}{n}$, in which C represents the number of cubic centimeters which must be added, N the number of cubic centimeters remaining after the test-titration, n the number of cubic centimeters consumed in one titration to bring about the end-reaction, and d the difference between the number of cubic centimeters used in one titration and that theoretically required. The amount of distilled water necessary for dilution is now added and the solution again tested, when 20 c.c. will correspond to 0.1 gramme of P_2O_5.

2. The acetic acid mixture is prepared by dissolving 100 grammes of sodium acetate in a little water, adding 30 grammes of glacial acetic acid and diluting the whole to 1000 c.c.

3. Tincture of cochineal. This may be prepared as follows : a few grammes of cochineal granules are digested at ordinary temperatures with 250 c.c. of a mixture of 3 volumes of water and 1 volume of 94 per cent. alcohol. The solution is then decanted and ready for use. The residue may be utilized in the preparation of a fresh supply of the tincture.

Application to the Urine.—Fifty c.c. of clear filtered urine are treated with 5 c.c. of the acetic acid mixture, the object being to transform any monacid sodium phosphate present into diacid sodium phosphate, and to neutralize any nitric acid that may be formed during the titration, as otherwise the nitric acid would cause a partial solution of the precipitated uranyl phosphate. A few drops of the tincture of cochineal are added, when the mixture is heated to the

boiling-point and titrated as described above. Two titrations are usually required.

Should it be desired to use potassium ferrocyanide as an indicator, the uranium solution must have been standardized with the same indicator, as errors will otherwise arise. The technique is simple. A number of drops of the potassium ferrocyanide solution (about 5 per cent.) are placed on a porcelain plate. After every addition of the uranium solution to the boiling urine a drop of the mixture is mixed on the plate with the ferrocyanide drop. The end reaction is indicated by the occurrence of a brown color.

The results are calculated as follows: supposing 15 c.c. of the uranium solution to have been used, the corresponding amount of P_2O_5 in 50 c.c. of urine is found from the equation : 20 : 0.1 : : 15 : x; and $x = 0.075$. The percentage-amount would, hence, be $0.075 \times 2 = 0.15$. Supposing the total amount of urine to have been 2000 c.c., the elimination of P_2O_5 would correspond to 3 grammes.

The presence of sugar and albumin does not interfere with the method.

Separate Estimation of the Earthy and Alkaline Phosphates.—If the alkaline and earthy phosphates are to be determined separately, the total amount of P_2O_5 is estimated in one portion of the urine, while the P_2O_5 in combination with the alkaline earths is determined in another, as follows:

Two hundred c.c. of filtered urine are made strongly alkaline with ammonium hydrate and set aside, covered, for several hours, when the earthy phosphates thus precipitated are collected on a filter, washed with dilute ammonia (1 : 3), and then transferred to a beaker, with the aid of a little water containing a few drops of acetic acid, by perforating the filter. They are then dissolved with as little acetic acid as possible, diluted to 50 c.c. with distilled water, and titrated with the uranium solution as described. The difference between the total amount of P_2O_5 and the amount thus obtained indicates the quantity of alkaline phosphates present.

Removal of the Phosphates from the Urine.—Whenever it is necessary to remove the phosphates from the urine in the course of an analysis, as is frequently the case, the urine is rendered alkaline by the addition of the hydrate of an alkaline earth and precipitated with a soluble calcium or barium salt. They may also be precipitated by means of neutral or basic lead acetate, in which case the excess of lead is removed by means of hydrogen sulphide or dilute sulphuric acid.

The Sulphates.

The sulphuric acid found in the urine is derived essentially from the albuminous material which is constantly broken down in the body, a very small portion only of the inorganic sulphates being

referable to the mineral constituents of the food. As was pointed out in the chapter on Reaction, sulphuric acid is constantly produced in the body, and, coming into contact with the so-called neutral phosphates present in almost all the tissues, transforms these into acid phosphates, according to the equation

$$2Na_2HPO_4 + H_2SO_4 = 2NaH_2PO_4 + Na_2SO_4,$$

both appearing in the urine. The alkaline carbonates, which are derived from the organic salts ingested by a process of oxidation, are also attacked by the sulphuric acid.

As the amount of food ingested is gradually diminished a point is reached when the body most tenaciously holds any alkaline salts that may still be present. A new source for the neutralization of the acid is then found in the ammonia, which would otherwise have been transformed into urea.

While the greater portion of the sulphuric acid excreted in the urine is found in the form of mineral sulphates, about one-tenth of the total amount may be shown to be in combination with aromatic substances belonging to the oxy-group; most important among these are the salts of phenol, indoxyl, and skatoxyl.

Indoxyl and skatoxyl, as will be shown later, are derived from indol and skatol, which, together with phenol, are formed during the process of intestinal putrefaction. Their amount increases and decreases with the degree of putrefaction, and hence serves as an index of its intensity.

The mineral sulphates have been termed preformed sulphates in contradistinction to the others, which are known as conjugate or ethereal sulphates. In the following pages the former will be designated by the letter A, the conjugate sulphates by the letter B, and the total sulphates as $A + B$.

The amount of $A + B$ excreted in the twenty-four hours by a normal individual varies between 2 and 3 grammes, the ratio of A to B being as 10 : 1.[1]

From what has been said, it is apparent that the elimination of sulphates is largely dependent upon the degree of albuminous decomposition taking place in the tissues and fluids of the body, and hence to a certain extent upon the quantity of proteid material ingested, the mineral sulphates occurring in such small amount in the food as scarcely to affect the quantity excreted. Secondarily, the degree of intestinal putrefaction plays a *rôle*. The excretion of $A + B$ is thus increased by a diet rich in animal proteids; the time after a meal, however, at which such an increase can be demonstrated varies greatly, depending essentially upon the time necessary for digestion. With a vegetable diet, on the other hand, the total sulphates will be found diminished. During starvation $A + B$ is, of course, also

[1] v. d. Velden, Virchow's Archiv, vol. vii. p. 343.

diminished, this diminution affecting A especially; but in some cases B may be considerably increased.[1]

An increase in the elimination of the total sulphates is observed, as would be anticipated, in all cases in which an increased tissue-destruction is taking place, as in the acute febrile diseases. It must be remembered, however, that the quantity excreted is then not always greater than during convalescence, the diet remaining the same. Here, as elsewhere, in urinary studies, it is necessary to distinguish between a relative increase and an absolute decrease. In pneumonia and acute myelitis the highest figures have been observed, the increased elimination during the febrile period being especially marked:[2]

	Fever diet.		Full diet.
	Fever.	No fever.	No fever.
Pneumonia	3.51 gm.	1.47 gm.	2.25 gm.
Acute myelitis	2.62 gm.	1.52 gm.	2.33 gm.

During convalescence the excretion of the sulphates is diminished, a retention analogous to that of the chlorides and phosphates taking place. In contradistinction to the latter salts, it is in all probability not the mineral matter proper that is demanded by the body, but the sulphur-containing albuminous material.

A considerable elimination of $A + B$ has also been observed in leukæmia, in which an average of 2.46 grammes is excreted, as compared with 1.51 grammes by a healthy individual receiving the same amount and kind of food. In one case of acute leukæmia 5.8 grammes were eliminated on the day preceding death.[3]

In diabetes mellitus, diabetes insipidus, œsophageal carcinoma, progressive muscular atrophy, pseudohypertrophic paralysis, and eczema an increased elimination has likewise been observed, while in chronic renal diseases a diminished excretion is the rule.

A study of the elimination of the *conjugate sulphates* and of the relation existing between A and B in disease is still more important than that of the total sulphates; but in both cases the data available are scanty, and further observations are urgently needed. v. Noorden regards the elimination of more than 0.3 gramme of conjungate sulphates, in the twenty-four hours, as excessive, the patient being on an ordinary mixed diet.

The conjugate sulphates, as would be expected, are increased in all cases of increased intestinal putrefaction. In coprostasis the result of carcinoma the ratio of the preformed to the conjugate sulphates, normally 10, may diminish enormously. In one case, reported by Kast and Baas, it fell to 2, but rose to 7 and 8, and finally to 9.5

[1] Clare, Inaug. Diss., Dorpat, 1854.
[2] P. Fürbringer, Virchow's Archiv, vol. lxxiii. p. 39.
[3] Ebstein, Deutsch. Arch. f. klin. Med., vol. xliv. p. 346.

and 15 after an artificial anus had been established.[1] I have myself observed a drop to 1.5 in a case of volvulus of ten days' standing. Biernacki[2] found an increase in the elimination of conjugate sulphates amounting to from 0.15 to 0.5 gramme pro die in cases of chronic parenchymatous nephritis, going hand in hand apparently with a decrease in the secretion of hydrochloric acid by the stomach; the normal amount, according to his observations, varies from 0.1973 to 0.2227 gramme. In one case *B* fell from 0.4382 to 0.1505 during the administration of hydrochloric acid, to increase again to 0.4127 upon its discontinuance.

In accord with these observations are those of Wasbutzki and Kast.[3] The former found an increased elimination of *B* in cases of intense bacterial fermentation taking place in the stomach, while hydrochloric acid was either totally absent or present in greatly diminished amount. A diminished elimination was observed in cases of intense torular fermentation, hyperchlorhydria existing at the same time. In the absence of hydrochloric acid a normal or even a slightly diminished amount was observed in cases of intense acid fermentation, lactic acid and butyric acid being present in large quantities. By neutralizing the gastric juice with large doses of sodium bicarbonate Kast was able to bring about a marked increase in the elimination of *B*, the ratio *A* : *B* having fallen from 10.3–16.1 to 2.9–6.1.

Personal observations have led me to the same conclusion, so that the following rules may be formulated :[4]

1. A diminution in the secretion of hydrochloric acid is accompanied by an increased degree of intestinal putrefaction.

2. An increase in the secretion of hydrochloric acid is usually accompanied by a decrease in the degree of intestinal putrefaction.

3. The degree of intestinal putrefaction may be measured directly by the elimination of the conjugate sulphates.

(See also the chapter on the Aromatic Bodies.)

In obstructive jaundice the excretion of *B* is likewise increased; it returns to the normal as soon as the permeability of the biliary passages has again become established. The total sulphates were found in diminished amount in cases of non-obstructive jaundice. In Böhm's[5] cases of catarrhal jaundice the excretion of conjugate sulphates varied between 0.4 and 0.7. gramme. Of interest in this connection are the observations of Müller[6] who notes the elimination of 0.29, 0.24, and 0.28 gramme of conjugate sulphates on three consecutive days in a case of total obstruction of the biliary duct in

[1] Kast u. Baas, Münch. med. Woch., 1888.
[2] Biernacki, Deutsch. Arch. f. klin. Med., vol. lxix.
[3] Kast, Festsch. z. Eröffnung d. neuen allgem. Krankenhauses, Hamburg, 1889. Wasbutzki, Arch. f. exper. Path. u. Pharmakol., vol. xxvi.
[4] C. E. Simon, Am. Jour. Med. Sci., 1895, vol. cx.
[5] A. Böhm, Deutsch. Arch. f. klin. Med., 1901, vol. lxxi. p. 73.
[6] Müller, Zeit. f. klin. Med., 1887, vol. xii.

consequence of a stone. The patient during this period was on a milk diet, and there can be little doubt that the low values are here referable to the germicidal properties of the milk. On a meat diet the same patient passed 0.48 and 0.51 gramme. Other observers have obtained less constant results in their cases of catarrhal jaundice. In cases of hepatic cirrhosis and malignant disease of the liver Eiger[1] and Hopadze[2] found increased amounts of conjugate sulphates.

In cases of diarrhœa $A + B$, as well as B, is diminished, while $A : B$ is increased.

Of drugs, large doses of morphin, potassium bromide, sodium salicylate, and antifebrin appear to cause an increased elimination of the total sulphates, while alcohol slightly diminishes the excretion.

Most important are the observations which have established a diminished excretion of the conjugate sulphates following ingestion of the terpenes and camphor, Karlsbad and Marienbad water, which latter two, however, at first cause an increase. Kefir, in doses of from 1 to 1.5 liters pro die, has proved a most excellent remedy with which to combat intestinal putrefaction. Injections of tannic acid and of a saturated solution of boric acid apparently produce little effect unless the dose is so large as to cause symptoms of poisoning.

Tests for the Sulphates in the Urine.—The detection of the preformed and the combined sulphates in the urine depends upon the fact that the sulphates of the alkalies are precipitated by barium chloride as insoluble barium sulphate, according to the equation:

$$K_2SO_4 + BaCl_2 = BaSO_4 + 2KCl.$$

In the urine the addition of barium chloride at the same time causes a precipitation of the phosphates. These must be kept in solution by the addition of an acid, acetic acid being employed for this purpose whenever the presence of the preformed sulphates is to be demonstrated; hydrochloric acid is inadmissible, as it would cause decomposition of the conjugate sulphates and set free the sulphuric acid thus held.

To test for the preformed sulphates, a few cubic centimeters of urine strongly acidified with acetic acid are treated with a few drops of a solution of barium chloride, when in their presence a cloud or a white precipitate of barium sulphate will occur.

To test for the conjugate sulphates, 25 c.c. of urine are treated with about the same volume of an alkaline barium chloride mixture (2 volumes of a solution of barium hydrate and 1 volume of a solution of barium chloride, both saturated at ordinary temperatures) and filtered after a few minutes, the preformed sulphates as well as

[1] Eiger, Inaug. Diss., St. Petersburg, 1893.
[2] Hopadze, Wratsch, 1893, Nos. 48–50.
[3] Zülzer, Unters. über d. Semiol. d. Harns, Berlin, 1884.

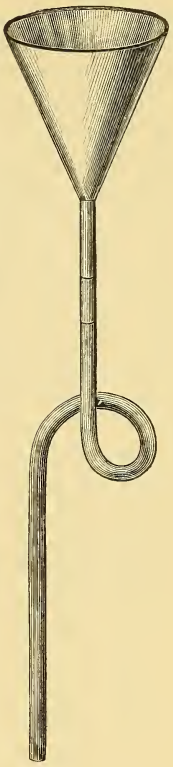
the phosphates being thus removed. The filtrate is then strongly acidified with hydrochloric acid and boiled; the occurrence of a precipitate is referable to conjugate sulphates.

Quantitative Estimation of the Sulphates.[1]—The principle of the method employed is the same as that just described, the preformed sulphates contained in the urine forming an insoluble precipitate of barium sulphate when treated directly with barium chloride, while the combined sulphates do so only after having been decomposed with strong hydrochloric acid with the application of heat. In order to estimate the amount of preformed and conjugate sulphates, it is best to determine the total sulphates in one portion, and the combined sulphates in another, the difference between the two giving the preformed sulphates.

Fig. 92.

A Gooch filter.

Fig. 93.



A suction-funnel.

Quantitative Estimation of the Total Sulphates.—One hundred c.c. of clear filtered urine are treated with 8 c.c. of hydrochloric acid (specific gravity 1.12) and heated to the boiling-point, when 20 c.c. of a hot saturated solution of barium chloride are added. The mixture is kept on a water-bath until the barium sulphate has settled down and the supernatant fluid appears clear; this usually requires about a half hour. The precipitate is now filtered off through a Schleicher and Schüll filter, or a Gooch filter (Fig. 92), provided with a close-fitting plug of asbestos, the whole having been previously dried and weighed. Care should be taken never to allow the filter to run dry, and small amounts of hot water must be added to the last cubic centimeters remaining, the final traces being placed upon the filter with the aid of a rubber-tipped glass rod. The precipitate is washed with boiling water until a specimen of the washings is no longer rendered cloudy, even on standing a few minutes after the addition of a drop of dilute sulphuric acid. Gum-like substances, as well as pigments, are removed by washing with hot alcohol (70 per cent.), and then filling the filter two or three times with ether. A suction apparatus is very convenient, but not neces-

[1] E. Salkowski, Zeit. f. physiol. Chem., 1886, vol. x. p. 346; and Virchow's Archiv, 1888, vol. lxxix. p. 551.

sary; a simple glass tube, bent upon itself, will answer the purpose (Fig. 93).

If a paper filter has been used, it is placed in a weighed platinum or porcelain crucible and ignited. The ash is then heated, at first moderately, and almost completely covered with the lid. It is then heated, only half covered, for from five to seven minutes, until the contents of the crucible are white. The crucible, when cooled, is placed in a desiccator and weighed, the difference between the first and the second weighing giving the weight of the barium sulphate obtained from 100 c.c. of urine.

A reduction of some of the sulphate usually takes place during the process of combustion, owing to the presence of organic matter, so that the weight obtained is actually too low. This error may be corrected in the following manner: the barium sulphate is washed into a small beaker with a small amount of water and titrated with a one-tenth normal solution of sulphuric acid, using phenolphthalein as an indicator. Each cubic centimeter of the one-tenth normal solution corresponds to 0.004 gramme of barium sulphate. The actual amount of sulphates contained in 100 c.c. of urine is ascertained by adding the figure thus found to that obtained by weighing (see below).

Instead of correcting as just described, the ash may be moistened with a few drops of a dilute solution of sulphuric acid; then when heat is applied again any sulphide that may have formed is transformed into the sulphate.

Quantitative Estimation of the Conjugate Sulphates.—One hundred c.c. of clear filtered urine are mixed with 100 c.c. of an alkaline solution of barium chloride (see above), the mixture being thoroughly stirred. After a few minutes it is filtered through a dry filter into a dry graduate to the 100 c.c. mark. This portion, corresponding to 50 c.c. of urine, is now strongly acidified with dilute hydrochloric acid and brought to the boiling-point. It is kept upon a boiling water-bath until the barium sulphate has settled and the supernatant fluid is clear. The precipitate is filtered off, washed, dried, and weighed, as described above. The weight thus obtained, multiplied by 2 and deducted from the amount found according to the first method, indicates the amount referable to the preformed sulphates. The molecular weight of $BaSO_4$ being 232.82, that of SO_3 79.86, of H_2SO_4 97.82, and of S 32, the figure expressing the amount of H_2SO_4, SO_3, or S, corresponding to 1 gramme of $BaSO_4$, is found according to the following equations:

$232.82 : 79.86 :: 1 : x$; and $x = 0.34301$. $\therefore$ 1 gramme of $BaSO_4 = 0.34301$ gramme of SO_3.

$232.82 : 97.82 :: 1 : x$; and $x = 0.42015$. $\therefore$ 1 gramme of $BaSo_4 = 0.42015$ gramme of H_2SO_4.

$232.82 : 32 :: 1 : x$; and $x = 0.13744$. $\therefore$ 1 gramme of $BaSO_4 = 0.13744$ gramme of S.

To calculate results, it is only necessary to multiply the weight of the $BaSO_4$ by 0.34301, 0.42015, or 0.13744, in order to ascertain the amount of sulphuric acid contained in 50 c.c. of urine, in terms of SO_3, H_2SO_4, or S, respectively.

Neutral Sulphur.

While the greater portion of the sulphur of the body is eliminated in an oxidized form, small amounts of non-oxidized sulphur bodies are likewise found in every urine. They are collectively spoken of as the neutral sulphur of the urine, and under normal conditions constitute from 12 to 15 per cent. of the total sulphur. The relation existing between the oxidized and the neutral form is, however, inconstant, and varies with the character of the diet, the degree of the proteid metabolism, etc.

Of the nature of the neutral sulphur bodies which occur in *normal* urine, comparatively little is known. At the present time we are acquainted with only two substances belonging to this order, viz., certain sulphocyanides and cysteïn, or a body which is closely related to it. The greater portion of *the sulphocyanides* is undoubtedly derived from the saliva that has been swallowed and absorbed, while a smaller amount may be referable to the trace which is said to be present in normal, uncontaminated gastric juice. The amount of sulphur which is present in this form represents about one-third of the total quantity of the neutral sulphur. *Cysteïn*, probably is an intermediary product of the normal metabolism of proteid material. Under normal conditions, however, the greater portion is oxidized to sulphuric acid, and traces only escape to be eliminated as such.

Whether or not *tauro-carbaminic acid*, which is a derivative of taurin, is a constant constituent of the urine, remains an open question, but is very probable. We know, as a matter of fact, that the amount of neutral sulphur undergoes a distinct diminution in animals when the bile is prevented from entering the intestinal canal by establishing an external fistula. Under pathological conditions a corresponding increase is observed in cases of biliary obstruction, and the amount of neutral sulphur may then reach 40 per cent. of the total sulphur.

Thiosulphates, which are normally present in the urine of dogs and cats, do not occur in human urine under normal conditions. That they may be present in disease has been shown by Strümpell, who found them in a case of typhoid fever. Further observations, however, are wanting.

Another sulphur body belonging to this class, which Abel discovered in the urine of dogs, and which appears to be identical with *ethyl sulphide*, has not as yet been found in the urine of man.

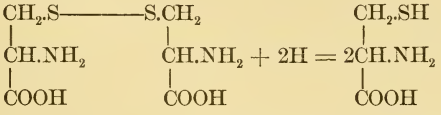
The greatest increase in the amount of the neutral sulphur is observed under certain conditions associated with the appearance of *cystin*. Normally this is not present in the urine, while traces of *cystëin*, or a closely related substance, as I have already stated, are found. Cystin is of albuminous origin, and as a matter of fact it has been ascertained that all of the loosely combined sulphur and even a portion of the firmly combined form exists in the albumins in the form of the cystin complex. According to Baumann and v. Udranszky, its appearance in the urine is closely connected with the formation of certain diamins, viz., cadaverin, putrescin, and a third diamin which is probably identical with saprin or neuridin. As these diamins were hitherto supposed to result only from the action of certain specific bacteria upon albuminous material, cystinuria was regarded as evidence of a definite infectious process. It is to be noted, however, that cystin itself does not occur in the feces, and that diaminuria does not necessarily accompany the cystinuria. As the result of personal observations I have been led to the conclusion that a causal connection does not exist between the two conditions, and that the diamins in question can be produced in the body-tissues directly without the intervention of micro-organisms. I regard cystinuria essentially as a metabolic anomaly, the result of a specific deficiency of the oxidation-processes taking place in the body. The condition may be temporary, but as a rule it is permanent. It may occur among several members of the same family, but it is noteworthy that no case has as yet been reported in which a parent and child both were cystinuric. Consanguinity among parents, which is not infrequently observed in cases of alkaptonuria, is the exception in cystinuria.

The amount of neutral sulphur which may be met with in cystinuria is subject to wide variation, but not infrequently exceeds 30 per cent. of the total sulphur. As a general rule, the amount of cystin eliminated in the twenty-four hours is less than 0.5 gramme. At times, however, larger quantities are found, and on one occasion I obtained more than 1 gramme. Clinically it is of interest in so far as its continued production may give rise to the formation of calculi.

Unless cystin occurs as a deposit, its presence will scarcely be suspected. The substance, however, may occur also in solution, and it not infrequently happens that attention is first drawn toward its existence in this state owing to the marked odor of hydrogen sulphide, which such urines develop on standing (see Hydrothionuria). If acetic acid is then added in excess, the characteristic hexagonal plates may crystallize out. The same result is obtained also by allowing the urine to undergo ammoniacal decomposition, as cystin is insoluble in solutions of ammonium carbonate.

Structurally cystin is the disulphide of cystëin which latter is

β-amido-thiolactic acid. On reduction it is transformed into cystëin according to the equation :



Cystin crystallizes in hexagonal plates which are quite characteristic, and not likely to be confounded with other crystalline elements that may be present in urinary sediments. If doubt should arise, their solubility in ammonia and hydrochloric acid, and their insolubility in acetic acid, water, alcohol, and ether, will lead to their identification.

The quantitative estimation of cystin is rather unsatisfactory, as no method is known which yields reliable results. On the whole, it is perhaps best to determine the neutral sulphur, and to refer the increase beyond its normal value to the presence of cystin.

Quantitative Estimation of the Neutral Sulphur in the Urine.—In 100 c.c. of urine the oxidized sulphur, viz., the mineral and the conjugate sulphates, are estimated as described on page 409. In the second portion the total sulphur then is determined, the difference indicating the amount of the neutral sulphur.

To determine the total amount of sulphur, 100 c.c. of urine are treated with 12 grammes of a mixture of sodium and potassium carbonate (11 : 14), and evaporated to dryness in a nickel crucible. The residue is fused thoroughly, allowed to cool, and extracted with hot water. The carbonaceous residue is filtered off and the filtrate and washings are treated with a few crystals of potassium permanganate. After heating for about fifteen minutes (more potassium permanganate should be added if during this time the solution becomes decolorized, when heat is again applied for fifteen minutes), concentrated hydrochloric acid is added until the reaction is distinctly acid. This solution is then brought to the boiling-point and treated with about 20 c.c. of a saturated solution of barium chloride. The barium sulphate thus formed is then collected and weighed as described on page 339. The difference between this result and the first indicates the amount of neutral sulphur.

The total amount of sulphur in the urine is still more conveniently determined according to the *method of Glaser*, as modified by Modrakowsky :[1] 1 or 2 grammes of sodium peroxide are placed in a nickel dish, and covered with 50 c.c. of urine, added drop by drop. The fluid is evaporated to a syrup on a water-bath, and further treated with 2–3 grammes of the peroxide, which is added slowly while stirring. As soon as the reaction, which at first is quite vigorous, has subsided somewhat, the dish is removed from the water-bath and heated with a small alcohol lamp. If necessary, 1–3

[1] Modrakowski, Zeit. f. phys. Chem., 1903, vol. xxxviii. p. 562.

grammes more of the peroxide are added. The mass now forms brown drops and finally becomes thick; this ends the reaction. On cooling, the fusion is dissolved in hot water; the solution is filtered and feebly acidified with hydrochloric acid. Barium chloride is then added and the process continued as described above (page 409).

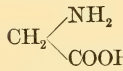
LITERATURE.—E. Salkowski, Virchow's Archiv, vol. lxvi. p. 313, and vol. cxxxvii. p. 381. Goldmann u. Baumann, "Zur Kenntniss der Schwefelhaltigen Verbindungen des Harns," Zeit. f. physiol. Chem., vol. xii. p. 254. E. Salkowski, Virchow's Archiv, vol. lviii. p. 461. J. Munk, Ibid., vol. lxix. p. 354; and Deutsch. med. Woch., 1877, No. 46. O. Schmiedeberg, "Ueber das Vorkommen von Unterschwefliger Säure im Harn," Arch. d. Heilk., vol. viii. p. 425. A. Strümpell, Ibid., vol. xvii. p. 390. J. Abel, "Ueber das Vorkommen von Ethylsulfid im Hundeharn," etc., Zeit. f. physiol. Chem., vol. xx. p. 253. (See also Cystinuria and Hydrothionuria.) C. E. Simon, "Cystinuria and its Relation to Diaminuria." Am. Jour. Med. Sci., 1900, vol. cxix. p. 39. C. E. Simon and M. W. Lewis, "Transitory Cystinuria," Ibid., 1902, vol. cxxiii. p. 838.

Urea.

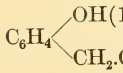
Urea is by far the most important nitrogenous constituent of the urine, and normally represents from 85 to 86 per cent. of the total amount of nitrogen which is eliminated by the kidneys. Chemically, it may be regarded as carbamide—*i. e.*, as the amide of carbonic acid—and is represented by the formula

It is thus a comparatively simple substance, and the question naturally arises: In what relation does urea stand to the highly complex albuminous molecule from which it is derived? Numerous hypotheses have been offered to explain this problem, but, although we are in possession of a number of very suggestive data, a final answer to the question cannot be given at the present time. In all likelihood, however, the urea may originate from the albumins in different ways.

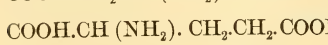
During the hydrolytic decomposition of the albumins by acids and alkalies bodies are constantly formed which belong to the class of amido-acids, and these bodies Schultzen and Nencki have accordingly regarded as intermediary products in the formation of urea within the tissues also. The most important members of this group are, leucin, tyrosin, glycocoll, asparaginic acid, and glutaminic acid. They are represented by the formulæ:

 —Glycocoll, or amido-acetic acid.

—Leucin, or amido-capronic acid.

 —Tyrosin, or para-oxy-phenyl-amido-propionic acid.

—Asparaginic acid, or amido-succinic acid.

 —Glutaminic acid, or amido-glutaric acid.

When introduced into the mammalian organism from without, the nitrogen of these bodies appears in the urine, to a large extent at least, as urea. An analogous formation from the tissue-albumins was hence also supposed to occur, but nothing is known of the manner of their transformation in the body into urea. Different possibilities suggest themselves. It is thus conceivable that cyanic acid—CONH—may be produced as an intermediary product, and that urea then results through the interaction of two molecules of the substance, *in statu nascendi*, according to the equation

$$CONH + CONH + H_2O = CO\begin{matrix}NH_2\\NH_2\end{matrix} + CO_2$$

On the other hand, a transformation of the amido-acids into the ammonium salts of the fatty acids standing next in order in the downward scale may also be imagined. Ammonium carbonate would then result, which, through loss of water, could give rise to urea. In the case of glycocoll this transformation could be represented by the following equations:

(1) $\underset{\text{Amido-acetic acid.}}{CH_2.NH_2.COOH} + 2O = \underset{\text{Ammonium formate.}}{NH_4.COOH} + CO_2$

(2) $\underset{\text{Ammonium formate.}}{2NH_4.COOH} + 2O = \underset{\text{Ammonium carbonate.}}{(NH_4)_2CO_3} + H_2O + CO_2$

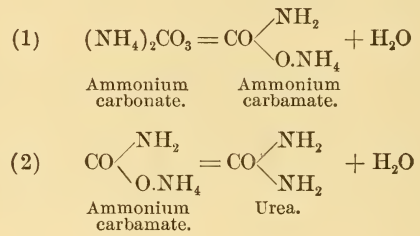
(3) $(NH_4)_2CO_3 = CO\begin{matrix}NH_2\\NH_2\end{matrix} + 2H_2O$

According to Drechsel, further, the amido-acids are transformed into carbonic acid, two molecules of which then unite to form urea, carbon dioxide, and water:

$$CO\begin{matrix}NH_2\\OH\end{matrix} + CO\begin{matrix}NH_2\\OH\end{matrix} = CO\begin{matrix}NH_2\\NH_2\end{matrix} + CO_2 + H_2O$$

The hypothesis of Schultzen and Nencki regarding the origin of urea from amido-acids is supported by the fact that these substances, when introduced into the mammalian organism from without, are largely transformed into urea during their passage through the body. It is known, moreover, that in certain diseases, such as acute yellow atrophy, the urea may disappear from the urine almost entirely, its place being taken by leucin and tyrosin. In other conditions, however, in which the formation of urea is even more seriously impaired, leucin and tyrosin do not appear in the urine, and there is a growing tendency among physiologists at the present time to

abandon the older view of Schultzen and Nencki, and to explain the apparently vicarious elimination of the amido-acids in acute yellow atrophy upon a different basis. Leucin and tyrosin are normally scarcely ever encountered in the mammalian organism, and the opinion now prevails that the greater portion of the nitrogen which is to be eliminated from the body leaves the tissues as the ammonium salt of paralactic acid. In the liver this is transformed into ammonium carbonate, from which urea then results synthetically, with the intermediary formation of ammonium carbamate. This transition may be represented by the equations:



This hypothesis has much in its favor. We thus find that after extirpation of the liver in geese the uric acid, which in birds plays the same part as the urea in mammals, disappears, and is largely replaced by ammonium lactate. In diseases of the liver, moreover, in which an extensive destruction of the parenchyma is taking place, as in some cases of acute yellow atrophy, in phosphorus poisoning, etc., the elimination of urea is diminished, and in its place a corresponding amount of ammonia in combination with lactic acid is found. In dogs in which the liver has been in part excluded from the general circulation by the establishment of an Eck-fistula, and in which the hepatic artery has at the same time been ligated, the elimination of urea is much diminished, while that of ammonia increases rapidly so soon as the first symptoms of illness appear in the animals. In such cases, owing to the incomplete isolation of the organ, ammonium carbamate appears in the urine, instead of ammonium lactate. From these observations it is apparent also that the synthesis of urea takes place in the liver. This is further proved by the fact that on transfusion of isolated livers of dogs with blood to which ammonium carbonate or ammonium lactate has been added, urea is formed as a result. In other organs of the body this synthesis apparently does not occur, but there is evidence to show that at least a small amount of urea originates elsewhere within the body through processes of hydrolysis. This amount, however, is unquestionably slight. That a fraction, moreover, is formed from uric acid, and in the last instance from the xanthin-

bases through processes of oxidation, can scarcely be doubted, but this transformation apparently also takes place in the liver.[1]

Before going on to a consideration of the quantitative excretion of urea in health and disease it will be well to form an idea of its ultimate sources. To this end, the theory of Voit[2] should be recalled, according to which, albuminous material exists in the body in two different forms—*i. e.*, as organized albumin, which is built up in the form of the tissues of the body, and as unorganized albumin or circulating albumin, which must be regarded in a manner as a reserve, to be used in tissue-repair or to be broken down if not used, and to be replaced by the proteids ingested with the next meal. It may hence be said that, as in the case of the mineral constituents of the urine, the urea is referable on the one hand to the proteids of the food, and on the other to the proteids of the body-tissues. It is clear then that elimination of urea will continue during starvation.

It has been stated that 84 to 86.6 per cent. of all the nitrogen eliminated in the urine is in the form of urea, the remaining 13.4 per cent. being excreted as uric acid, hippuric acid, kreatinin, xanthin-bases, etc. It might hence be supposed that an accurate idea of the degree of tissue-destruction could be formed from a quantitative estimation of urea. This, however, is not the case, and especially in pathological conditions, as the quantitative relations existing between the excretion of urea and the remaining nitrogenous constituents are subject to wide variation. In acute yellow atrophy, for example, as pointed out above, urea may disappear entirely from the urine, the nitrogen being eliminated in the form of other compounds. Whenever it becomes desirable then to gain an accurate insight into the degree of proteid-destruction or proteid-assimilation —in other words, into the nitrogenous metabolism—taking place in the body, it is necessary to resort to a quantitative determination of the total amount of nitrogen excreted by the kidneys ; the quantity found is then conveniently expressed in terms of urea. At the same time it is customary to express the amount of proteid tissue which is destroyed, as muscle-tissue, as this serves as a fair type of body-tissue in general.

As 100 grammes of lean muscle-tissue contain about 3.4 grammes of nitrogen, corresponding to 7.286 grammes of urea, 1 gramme of the latter is equivalent to 13.72 grammes of muscle-tissue. It is,

[1] The origin of urea: O. Schultzen u. M. Nencki, Zeit. f. Biol., 1872, vol. viii. p. 124. E. Salkowski, Zeit. f. physiol. Chem., 1879, vol. iv. p. 100. v. Knieriem, Zeit. f. Biol., 1874, vol. x. p. 279. E. Salkowski, Zeit. f. physiol. Chem., 1877, vol. i. p. 38. Hoppe-Seyler, Physiol. Chem., 1881, p. 810. Drechsel, Jour. f. prakt. Chem., vol. xv. p. 417, vol. xvi. pp. 169 and 180, and vol. xxii. p. 476. M. Hahn, V. Massen, M. Nencki, and J. Pawlow, "La fistula d'Eck," etc., Arch. d. Sci. biol. de St. Petersburg, 1892, vol. i.

Seat of formation: W. v. Schröder, Arch. f. exper. Path. u. Pharmakol., 1882, vol. xv. p. 364. W. Salomon, Virchow's Archiv, 1884, vol. xcvii. p. 149. Minkowski, "Ueber d. Einfluss d. Leberextirpation auf d. Stoffwechsel," Arch. f. exper. Path. u. Pharmakol., 1886, vol. xxi. p. 41, and 1893, vol. xxxi. p. 214.

[2] C. Voit, Physiol. d. allg. Stoffwechsels u. d. Ernährung. Herman's Handbuch d. Physiol., 1881, vol. vi. I. p. 301.

hence, only necessary to multiply the quantity of urea eliminated in the twenty-four hours, corresponding to the total amount of nitrogen found, by 13.72, in order to obtain an idea of the extent of albuminous destruction taking place in the body. If accurate results are desired, it becomes necessary to determine also the amount of nitrogen eliminated in the feces, a knowledge of the quantity in the food ingested being, of course, presupposed.

With all these data given, the nitrogenous metabolism of the body can be accurately controlled.

Example.—A patient eliminates 50 grammes of urea in twenty-four hours; these 50 grammes correspond to 50×13.72—*i. e.*, 686 grammes of lean muscle-tissue; on the other hand, he ingests an amount of nitrogenous material corresponding to only 10 grammes of urea, equivalent to 10×13.72—*i. e.*, 137.2 grammes of muscle-tissue. The difference between the amount ingested and that excreted in this case—*i. e.*, 548.8 grammes—must be referable to the destruction of organized albumin.

When the amount of nitrogen eliminated is equivalent to that ingested, *nitrogenous equilibrium* is said to exist. A healthy person is approximately in this condition.

It has been pointed out that during starvation urea is still eliminated from the body, although in diminished amount. The question now arises, What happens if at this time an amount of nitrogenous food is given which corresponds exactly in amount to that eliminated? Under such conditions an increased elimination of nitrogen takes place, all of the nitrogen ingested, in addition to that resulting from a breaking down of body-tissues, being excreted. The amount of nitrogen referable to the latter source, however, is somewhat less than that eliminated in the total absence of food. Unless starvation has been pushed too far, the body accommodates itself to the amount of food thus given and nitrogenous equilibrium is restored. If more food is allowed, an increased elimination results, which again leads to a condition of nitrogenous equilibrium, different levels, so to speak, being possible. This is well illustrated by comparing the condition of the poorly nourished North German laboring population with that of the well-fed merchants, the excretion of urea in the former amounting to 17.5 to 33.5 grammes, and in the latter to 30 or even 40 grammes.

It is apparent, then, that the elimination of urea, and of nitrogen in general, is subject to great variation, depending upon the amount ingested and *that* resulting from tissue-destruction, which in turn is influenced largely by the body-weight. A statement in figures expressing the daily elimination of urea and of nitrogen would, hence, be of very little value, especially in pathological conditions, in which the amount of nitrogen ingested is frequently very small. The elimination of nitrogen should hence always be compared with

the amount ingested, for which purpose the tables of König[1] will be found most convenient. At the same time it must be remembered that not all the nitrogen taken into the body as food undergoes resorption, and that a variable amount, which in disease may be considerable, is eliminated with the feces, so that in accurate work this nitrogen also must be taken into account. In order to obviate the tedious estimation of nitrogen in the feces, it has been proposed to determine the standard amount of urea which should appear in the urine of a healthy person under different forms of diet. Such experiments, of course, presuppose the control-person to be in a condition of nitrogenous equilibrium, which, from what has been said above, is readily accomplished, as the human body adapts itself with ease to different forms of diet. In private practice, however, such a procedure would be difficult, but here approximate results can be obtained from a parallel estimation of the chlorides. In health the elimination of the chlorides may be placed at about one-half of the urea. Whenever the nitrogen resulting from tissue-destruction is in excess of that referable to the proteids ingested, this relation between the excretion of chlorides and urea will be disturbed, as the tissues of the body contain very little sodium chloride. Whenever the amount of urea is in excess of the normal amount of chlorides, as indicated above, an increased tissue-destruction may be inferred, and *vice versa*. If, on the other hand, the chlorides are present in diminished amount, the conclusion may be drawn that a retention of albumins is taking place in the body; this is observed frequently during convalescence from acute febrile diseases.

An *increase in the amount of urea*, and, as a matter of fact, of all the nitrogenous constituents, is observed especially in the acute febrile diseases, notwithstanding the diminished ingestion of nitrogenous material, and is due to the greatly increased tissue-destruction.[2] An excretion of 50 grammes or more is here frequently observed. Formerly it was thought that the fever itself was responsible for this increased elimination. But this view became untenable when it was shown that the excretion of urea in the beginning of a febrile attack is not proportionate to the height of the temperature, reaching its highest point only when the fever has been continuous for several days. Still larger amounts, moreover, may be eliminated when the fever is abating. Similar observations have since been made. An increased elimination of nitrogen may also be noted in almost every case of ague preceding the onset of the fever. The latter, therefore, cannot be the only factor which causes the increased excretion of urea, and it has been suggested that the cells of the body have lost the power of taking up nitrogen. The question, however, whether this is dependent upon the increase

[1] J. König, Chemie d. menschlichen Nahrungs u. Genussmittel, Berlin, 1893.

[2] Vogel, Zeit. f. rationelle Med., N. F., vol. iv. p. 362. Huppert, Arch. d. Heilk., vol. vii. p. 1. Löbisch, Wien. med. Presse, 1889, vol. xxxix. p. 1521. Huppert u. Riesellt, Arch. d. Heilk., vol. x. p. 329. Bauer u. Künstle, Deutsch. Arch. f. klin. Med., vol. xxiv. p. 53.

in temperature or the action of certain toxic substances circulating in the blood, or upon both, still remains unanswered.

The large increase in the elimination of nitrogen in febrile diseases is especially striking in those which end by crisis. This is notably the case in pneumonia, in which it may persist for two or three days after the occurrence of the crisis. The assumption of an underlying insufficiency on the part of the cells furnishes a very satisfactory explanation for the continued increased elimination of urea. An increase beyond the amount eliminated during the febrile stage is possibly owing to a retention analogous to that of the mineral constituents of the urine.

Apparently, the only exception to the rule that the amount of urea is increased in acute febrile diseases, is acute yellow atrophy, in which the excretion of urea is not only greatly diminished, but may cease altogether, its place being taken by other nitrogenous bodies, such as ammonium lactate, leucin, and tyrosin.

Among afebrile diseases in which an increased elimination of urea has been noted, may be mentioned the ordinary forms of diabetes mellitus, in which the highest figures have been obtained, viz., 150 grammes or more pro die. This is, in all probability, explained, in part at least, by the ingestion of excessive amounts of proteid food by such patients, but carefully conducted experiments seem to show that a not inconsiderable portion of the urea is directly referable to increased tissue-destruction. The cases described by Hirschfeld,[1] however, which will be considered later on, form an exception to this rule.

An increase is observed also in dyspnœic conditions, and particularly in pneumonia, in which it is most marked on the day following the greatest difficulty in breathing. These observations, however, are not free from objections, as an increase has also been noted in conditions of apnœa.

v. Noorden and Lipman-Wolff have shown that anæmia as such is not necessarily associated with a pathological increase in the albuminoid metabolism. But it appears that in pernicious anæmia, at least in the bothriocephalus form, there are periods in which an increased albuminous disintegration does occur. According to Rosenqvist,[2] this is far too extensive to be dependent entirely upon the destruction of red corpuscles, but must be associated with changes in other nitrogenous tissues of the body. After the expulsion of the worms a well-marked nitrogenous retention was observed. Similar results were obtained in cases of cryptogenetic pernicious anæmia, where periods of marked increase of albuminoid disintegration alternated sometimes with such of distinct nitrogenous retention. Rosenqvist concludes that his observations are strongly in

[1] F. Hirschfeld, "Ueber eine neue klin. Form. d. Diabetes," Zeit. f. klin. Med., vol. xix. pp. 294 and 325.

[2] Rosenqvist, Berlin. klin. Woch., 1901, vol. xxxviii. p. 666.

support of the theory that cryptogenetic pernicious anæmia, like the bothriocephalus form, is also a toxic anæmia.

A moderate increase has been found in severe cases of leukæmia, scurvy, minor chorea, and paralysis agitans. Observations made in cases of hystero-epilepsy have given rise to conflicting results. It is claimed, on the one hand, that the excretion of urea is diminished following convulsive seizures of a hystero-epileptic nature, in contradistinction to an increased elimination following true epileptic attacks.

In cases of functional albuminuria associated with an increased elimination of uric acid or oxalic acid, or of both, as well as in numerous cases of gastro-intestinal disease, I have observed an increased elimination of urea, and believe that in the treatment of these diseases a systematic study of the excretion of nitrogen is of fundamental importance.

Of drugs, an increased elimination is produced by coffee, caffein, morphin, codein, ammonium chloride, sodium and potassium chlorides, lithium carbonate, following the ingestion of large amounts of water, etc. The data concerning the action of quinin, salicylic acid, cold baths, etc., are conflicting. A large increase has been observed in cases of phosphorus poisoning.

Electricity also appears to exert a marked influence upon the excretion of urea, producing an increased elimination.

The *diminished elimination of urea* observed in certain diseases of the liver,[1] notably in acute yellow atrophy, carcinoma, cirrhosis, and even in Weyl's disease, is of especial interest, and is in perfect accord with the theory that the liver is the main seat of its production.

As has been stated, urea may disappear altogether from the urine in acute yellow atrophy and also in Weyl's disease, notwithstanding the frequently not inconsiderable degree of fever. In cirrhosis, hyperæmia of the portal system has been thought to cause the diminution, which may be increased further in some cases by the occurrence of ascites. In short, the factors which may be regarded as causing a diminished elimination of urea in hepatic diseases may be summarized under the following headings:

1. Destruction of hepatic parenchyma.
2. Diminished velocity of the flow of blood through the liver.
3. Insufficient excretion of bile and coincident digestive disturbances.

Whenever there is disease affecting that portion of the renal parenchyma which is concerned especially in the elimination of urea, a diminished amount will, of course, be met with, and carefully conducted observations upon the excretion of the various urinary constituents are here of considerable value from a diagnostic as well as a therapeutic standpoint. As the glomeruli of the kid-

[1] Hallerworden, Arch. f. exper. Path. u. Pharmakol., vol. xii. Weintraud, Ibid., vol. xxxi. Stadelmann, Deutsch. Arch. f. klin. Med., vol. xxxiii. Fawitzki, Ibid., vol. xlv. Fränkel, Berlin. klin. Woch., 1878 and 1892. v. Noorden, Lehrbuch d. Path. d. Stoffwechsels, p. 287.

neys are mainly concerned in the elimination of water and salts from the blood, and as the striated epithelium of the convoluted tubules appears to provide for the excretion of urea, the elimination of a fair amount of the latter with a diminished elimination of salts, the phosphates being of especial interest, as they are derived to a large extent from albuminous material, would point more particularly to glomerular disease. On the other hand, a fair excretion of phosphates and a diminished excretion of urea would be indicative of tubular disease. Whenever glomeruli and tubuli contorti are equally diseased an insufficient elimination of both phosphates and urea will be observed.

While, as a rule, the excretion of urea is greatly increased in diabetes mellitus, certain cases, which have been elaborately described by Hirschfeld,[1] must be excepted. His researches have established beyond a doubt that the resorption of nitrogenous material from the intestines may be very much below normal, and with it the elimination of urea. Upon these grounds he has advocated the recognition of a distinct form of diabetes, which is characterized by a comparatively rapid course, the occurrence of colicky abdominal pains before or at the onset of the diabetic symptoms proper, the existence of pancreatic lesions in a certain proportion of the cases, a more moderate degree of polyuria, etc.

In mental diseases a diminished excretion of urea has been observed in melancholia and in the more advanced stages of general paresis, while an increase is associated with the increased ingestion of food during the first stage of profound dementia.

Following epileptic, cataleptic, and hysterical seizures, as well as in pseudohypertrophic paralysis, a decrease has been noted by some observers.

The diminished excretion observed in Addison's disease has also been regarded as of nervous origin.

All forms of chronic, non-progressive anæmia are associated with a decrease, as are also osteomalacia, impetigo, lepra, chronic rheumatism, etc. In chronic lead poisoning the elimination of urea may be greatly diminished.

Little is known of the influence of drugs in bringing about a diminished excretion of urea.

In conclusion, the relation existing between phosphatic excretion and that of nitrogen should be especially noted, for a consideration of which, see page 399.

Properties of Urea.—Urea crystallizes in two forms, viz., in long, fine, white needles if rapidly formed, or in long, colorless, quadratic rhombic prisms when allowed to crystallize gradually from its solutions.

At 100° C. it begins to show signs of decomposition; at 130° to 132° C. it melts; and when heated still further it is decomposed into cyanic acid and ammonia, of which the former is immediately trans-

[1] Loc. cit.

formed into its polymeric compound, cyanuric acid. The reaction which takes place is represented by the equations:

$$(1)\quad CO\begin{matrix}NH_2\\NH_2\end{matrix} = CONH + NH_3.$$

$$(2)\quad 3CONH = C_3O_3N_3H_3.$$

Biuret is formed as an intermediary product during this decomposition, 2 molecules of urea yielding 1 molecule of ammonia and 1 molecule of biuret, as represented in the equation

$$\begin{matrix}CO\begin{matrix}NH_2\\NH_2\end{matrix}\\CO\begin{matrix}NH_2\\NH_2\end{matrix}\end{matrix} = \begin{matrix}CO\begin{matrix}NH_2\\\end{matrix}\\CO\begin{matrix}NH\\NH_2\end{matrix}\end{matrix} + NH_3.$$

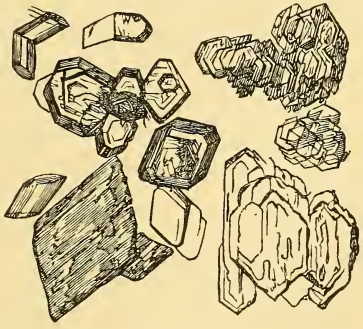
As this substance, obtained on dissolving the residue remaining after all the ammonia has been driven off by careful heating, yields a beautiful reddish-violet color when a drop or two of a very dilute solution of cupric sulphate is added to its solution alkalinized with sodium hydrate, this reaction may be employed as a test in the detection of urea (Biuret test).

Urea is readily soluble in water, fairly so in alcohol, and insoluble in anhydrous ether and benzol. The aqueous solution of urea is neutral in reaction, but this substance combines with acids, bases, and salts to form molecular compounds.

Of special interest are the compounds of urea with nitric acid, oxalic acid, and mercuric nitrate.

Urea nitrate, $CON_2H_4.HNO_3$, crystallizes in two different forms: in thin rhombic or six-sided colorless plates, which are frequently

FIG. 94.



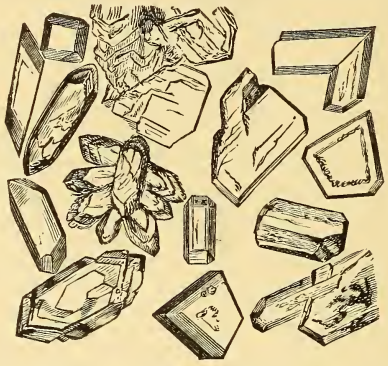
Urea nitrate crystals. (KRUKENBURG, after KÜHNE.)

observed arranged like shingles one on top of the other when rapidly formed (Fig. 94), while larger and thicker rhombic columns or plates are obtained if the process of crystallization is allowed to proceed

more slowly. Urea nitrate is readily soluble in distilled water, while in alcohol and in water containing nitric acid it dissolves with difficulty. Upon heating, it evaporates without leaving a residue.

Urea oxalate, $CON_2H_4.C_2H_2O_4$, crystallizes in rhombic or six-sided prisms or plates (Fig. 95), which are less soluble in water than the nitrate; in alcohol and in water containing oxalic acid it is only imperfectly soluble.

FIG. 95.



Urea oxalate crystals. (KRUKENBERG, after KÜHNE.)

With mercuric nitrate urea forms three different compounds, according to the concentration of the two solutions, viz., $(CON_2H_4)Hg_2(NO_3)_4$, $(CON_2H_4).Hg_3(NO_3)_6$, and $(CON_2H_4)_2.Hg(NO_3)_2 + 3HgO$. The latter compound is of special importance, as Liebig's quantitative estimation of urea was based upon its formation. It results when a 2 per cent. solution of urea is treated with a dilute solution of mercuric nitrate, the reaction taking place according to the equation

$$2CON_2H_4 + 4Hg(NO_3)_2 + 3H_2O = [2(CON_2H_4)_2Hg(NO_3)_2 + 3HgO] + 6HNO_3.$$

Very important is the behavior of urea when treated with a solution of sodium hypochlorite or hypobromite, the most usual method of estimating urea being based upon this reaction, which may be represented by the equation

$$CON_2H_4 + 3NaOBr = 3NaBr + 2N + CO_2 + 2H_2O.$$

In the chapter on Reaction it was pointed out that urine gradually undergoes ammoniacal decomposition when exposed to the air, and that this process is due to the action of a non-organized ferment; the ammonia is liberated according to the equation

$$CO\begin{matrix}HH_2\\ \\ NH_2\end{matrix} + H_2O = 2NH_3 + CO_2.$$

This decomposition may also be effected by heating a watery solution of urea in a sealed tube to 100° C

Separation of Urea from the Urine.—Fifty to 100 c.c. of urine are evaporated to a syrupy consistence upon a water-bath, and extracted with 100 to 150 c.c. of strong alcohol, by rubbing up the residue, while still hot, with the alcohol. Upon cooling, the mixture is filtered, the alcohol evaporated, and the residue treated with pure cold nitric acid. Urea nitrate then separates out either immediately or on standing. After twenty-four hours the crystalline mass is collected on a muslin filter, well strained, and freed from liquid by placing it upon plates of clay. The material is then dissolved in hot water, and the solution, if strongly colored, gently warmed with animal charcoal and filtered. This solution is neutralized with barium carbonate, and rendered alkaline with barium hydrate. The urea nitrate is thus decomposed, barium nitrate and urea being formed :

$$2CON_2H_4.HNO_3 + BaCO_3 = 2CON_2H_4 + Ba(NO_3)_2 + H_2O.$$

The barium is now removed by passing a stream of carbon dioxide through the solution and filtering off the precipitate. The filtrate is evaporated until any barium nitrate still remaining crystallizes out. This is removed by decantation, when upon further evaporation the urea crystallizes out, and may be dried between layers of filter-paper and recrystallized from 95 to 98 per cent. alcohol. The crystals thus formed may now be subjected to further tests. To this end, a few drops of an aqueous solution are added to a few cubic centimeters of a sodium hypobromite solution, when in the presence of urea bubbles of gas will be given off. With a solution of sodium hypochlorite the same result may be obtained, but in this case the evolution of gas takes place only upon the application of heat. The formation of biuret may also be demonstrated by carefully melting a few of the crystals in a test-tube, dissolving the residue when cool in a little water, and alkalinizing the solution with a little sodium hydrate; upon the addition of a dilute solution of cupric sulphate a beautiful reddish-violet color will develop, owing to the presence of biuret.

The addition of oxalic or nitric acid to a solution of urea will give rise to the formation of urea nitrate and oxalate, as described above.

This latter test may very conveniently be made under the microscope. A drop of the concentrated solution is placed upon a slide, covered, and a drop of pure nitric acid added from the side. Crystals of urea nitrate will then be seen to separate out, and may be recognized by their characteristic shingle-like arrangement (see Fig. 94).

When a urine is very rich in urea the mere addition of nitric acid will cause a more or less abundant precipitation of urea nitrate, and with this simple test an idea may even be formed of the amount present. An appearance of hoar-frost is thus noted when not less

than 25 grammes are present in the liter, while the formation of spangles of urea nitrate requires the presence of at least 45 grammes, and an abundant sediment occurs when 50 grammes or more are present.

Quantitative Estimation of Urea.—**Hypobromite Method.**—The method most commonly used in the clinical laboratory is the one based upon the decomposition of urea into carbon dioxide and nitrogen in the presence of sodium hypobromite. The reaction takes place according to the equation

$$CON_2H_4 + 3NaOBr = NaBr + CO_2 + 2H_2O + 2N.$$

The carbon dioxide thus formed is absorbed by an excess of sodium hydrate added to the hypobromite solution, while the nitrogen is set free, and can be collected and measured; the determination of the corresponding amount of urea is then a simple matter.

The only solution that is necessary is one of sodium hypobromite containing an excess of sodium hydrate. A 30 per cent. solution of the latter should be kept on hand and the sodium hypobromite solution prepared when required. To this end, 70 c.c. of the sodium hydrate solution are diluted with 180 c.c. of water and treated with 5 c.c. of bromine in a bottle provided with a ground-glass stopper, the mixture being thoroughly shaken until every trace of free bromine has disappeared. The sodium hypobromite solution, if kept in a perfectly dark and cool place, may be preserved for a week or two. The reaction which takes place between the sodium hydrate and the bromine may be represented by the equation

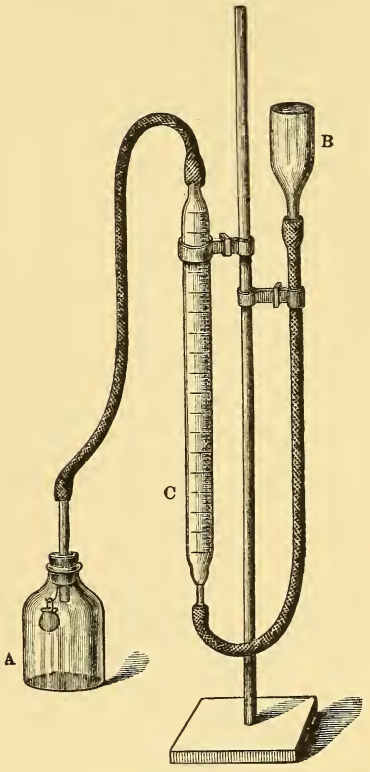
$$2NaOH + 2Br = NaBr + NaOBr + H_2O.$$

Various forms of apparatus, termed *ureometers*, have been suggested for the estimation of urea by this method. One which I have found very satisfactory is represented in Fig. 96. It consists essentially of a burette, C, with an ascending rubber tube attached to the reservoir B, which can be raised or lowered as required for the purpose of equalizing the pressure after collection of the gas. A descending tube leads to a wide-mouthed bottle, A, which contains the hypobromite solution. This is closed by a tightly fitting rubber stopper, to which a loop of platinum wire is attached carrying a little bucket made of glass or porcelain; this can be swung from its support by inclining the bottle.

Method.—The rubber stopper is removed from the bottle A, and water poured into B until the system BCA is filled to such an extent that the water-level is visible in B above the point where the rubber tube is attached. About 25 to 30 c.c. of the hypobromite solution are placed in the bottle A, and 2 c.c. of urine in the bucket; this is then attached to the wire loop. The stopper is now

carefully adjusted and the water in B and C brought to the same level, when the first reading is taken. A is then inclined until the bucket drops into the liquid below. The nitrogen which is liberated collects in the burette C; as a consequence the water falls in C and rises in B. After twenty to thirty minutes the pressure in C is equalized by lowering B until the water in both tubes is at the same level. The second reading is then taken, the difference between the two indicating the volume of nitrogen liberated from 2 c.c. of urine at the temperature of the water in CB, which, as well as the barometric pressure, should be previously noted.

Fig. 96.



The author's ureometer.

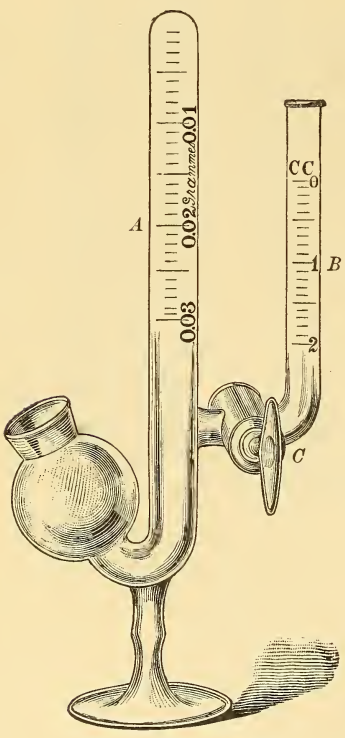
As the volume of gases is greatly influenced by the temperature, the barometric pressure, and the tension of the aqueous vapor, it becomes necessary, in order that the results reached shall be comparable with those obtained by other observers, to reduce the volume of nitrogen actually noted to a certain standard. This has been placed at 0° C. and 760 mercury millimeters pressure, in the absence of moisture. This correction is made according to the following formula:

$V = \frac{v.(B - T)}{760.(1 + 0.00366.t)}$, in which V represents the corrected volume of the gas in terms of c.c., v the volume actually observed, B the barometric pressure in Hgmm., T the tension of the aqueous vapor at the temperature noted, t. The volume of nitrogen observed being thus corrected, the calculation of the corresponding amount of urea is based upon the following considerations: from the formula CON_2H_4 it is apparent that 2 atoms of nitrogen are contained in 1 molecule of urea; in other words, that 28 parts by weight of nitrogen correspond to 60 parts by weight of urea. The equivalent of 1 gramme of urea is then found according to the equation: $60 : 28 : : 1 : x$; and $x = 0.46666$. The volume corresponding to 0.4666 gramme of dry nitrogen at 0° C. and 760

Hgmm. pressure is 372.7 c.c. It has been found, however, that only 354.3 c.c. of nitrogen are evolved from 1 gramme of urea at best when the hypobromite method is employed. Knowing that 354.3 c.c. of nitrogen correspond to 1 gramme of urea, the amount of urea to which the volume of nitrogen actually observed is referable would then be found according to the equation

$1 : 354.3 :: x : y$; and $x = \frac{y}{354.3}$, in which y denotes the number of cubic centimeters of nitrogen evolved from 2 c.c. of urine, and x the corresponding amount of urea. In order to ascertain the percentage-amount of urea it is only necessary to multiply the figure just obtained by 50.

Fig. 97.



Doremus' ureometer.

Precautions : 1. The urine must be free from albumin. 2. It should contain only about 1 per cent. of urea—*i. e.*, not more than 0.025 gramme in 2 c.c. Whenever a greater amount is noted, therefore, the urine is diluted to the proper degree, due allowance being made in the calculation.

In ordinary clinical work the barometric pressure, as well as the tension of the aqueous vapor, may be ignored, and in the accompanying tables the corresponding amount of urea may be directly read off at the temperatures 5°, 10°, 15°, 20°, 25°, and 30° C.

Of other forms of apparatus, the ureometers devised by Doremus, Green, Marshall, Hüffner, and Squibb may be mentioned.

The latest modification of Doremus' apparatus is certainly most convenient, and can be highly recommended. Its general construction is seen in Fig. 97. A small amount of urine is poured into B while the stopcock (C) is closed. This is then opened for a moment and again closed, so as to fill its lumen. The tube A is washed out with water and filled with the hypobromite solution. The tube B is filled with urine, and 1 c.c. (or less, if the urine is concentrated) is allowed to mix with the hypobromite solution in A. After all bubbles of gas have disappeared the reading is taken. The degrees marked upon the tube indicate

Urea. Table for a Temperature of 5° C.

	1	$\frac{1}{10}$	$\frac{2}{10}$	$\frac{3}{10}$	$\frac{4}{10}$	$\frac{5}{10}$	$\frac{6}{10}$	$\frac{7}{10}$	$\frac{8}{10}$	$\frac{9}{10}$
1	1.32	1.45	1.58	1.71	1.85	1.98	2.11	2.24	2.37	2.51
2	2.64	2.77	2.90	3.03	3.17	3.30	3.43	3.56	3.69	3.83
3	3.96	4.09	4.22	4.36	4.49	4.62	4.75	4.88	5.02	5.15
4	5.28	5.41	5.54	5.68	5.81	5.94	6.07	6.20	6.34	6.47
5	6.60	6.73	6.87	7.00	7.13	7.26	7.39	7.53	7.66	7.79
6	7.92	8.05	8.19	8.32	8.45	8.58	8.71	8.85	8.98	9.11
7	9.24	9.38	9.51	9.64	9.77	9.90	10.04	10.17	10.30	10.43
8	10.56	10.70	10.83	10.96	11.09	11.22	11.36	11.49	11.62	11.75
9	11.89	12.02	12.15	12.28	12.41	12.55	12.68	12.81	12.94	13.07
10	13.21	13.34	13.47	13.60	13.73	13.87	14.00	14.13	14.26	14.39
11	14.53	14.66	14.79	14.92	15.06	15.19	15.32	15.45	15.58	15.72
12	15.85	15.98	16.11	16.24	16.38	16.51	16.64	16.77	16.90	17.04
13	17.17	17.30	17.43	17.57	17.70	17.83	17.96	18.09	18.23	18.36
14	18.49	18.62	18.75	18.89	19.02	19.15	19.28	19.41	19.55	19.68
15	19.81	19.94	20.08	20.21	20.34	20.47	20.60	20.74	20.87	21.00
16	21.13	21.26	31.40	21.53	21.66	21.79	21.92	23,06	22.19	22.32
17	22.45	23.59	22.72	22.85	22.98	23.11	23.25	23.38	23.51	23.64
18	23.77	23.91	24.04	24.17	24.30	24.43	24.57	24.70	24.83	24.96
19	25.10	25.23	25.36	25.49	25.62	25.76	25.89	26.02	26.15	26.28
20	26.42	26.55	26.68	26.81	26.94	27.08	27.21	27.34	27.47	27.60
21	27.74	27.87	28.00	28.13	28.27	28.40	28.55	28.66	28.79	28.93
22	29.06	29.19	29.32	29.45	29.59	29.72	29.85	29.98	30.11	30.25
23	30.38	30.51	30.64	30.78	30.91	31.04	31.17	31,30	31.44	31.57
24	31.70	31.83	31.96	32.10	32.23	32.36	32.49	32.62	32.76	32.89
25	33.02	33.15	33.29	33.42	33.55	33.68	33.81	33.95	34.08	34.21
26	34.34	34.47	34.61	34.74	34.87	35.00	35.13	35.27	35.40	35.53
27	35.66	35.80	35.93	36.06	36.19	36.32	36.46	36.59	36.72	36.85
28	36.98	37.12	37.25	37.38	37.51	37.64	37.78	37.91	38.04	38.17
29	38.31	38.44	38.57	38.70	38.83	38.97	39.10	39.28	39.36	39.49
30	39.63	39.76	39.89	40.02	40.15	40.29	40.42	40.55	40.68	40.81

Urea. Table for a Temperature of 10° C.

	1	$\frac{1}{10}$	$\frac{2}{10}$	$\frac{3}{10}$	$\frac{4}{10}$	$\frac{5}{10}$	$\frac{6}{10}$	$\frac{7}{10}$	$\frac{8}{10}$	$\frac{9}{10}$
1	1.31	1.43	1.56	1.69	1.82	1.95	2.08	2.21	2.34	2.47
2	2.60	2.73	2.86	2.99	3.12	3.25	3.38	3.51	3.64	3.77
3	3.90	4.03	4.16	4.29	4.42	4.55	4.68	4.81	4.94	5.07
4	5.20	5.33	5.46	5.59	5.72	5.85	5.98	6.11	6.24	6.37
5	6.50	6.33	6.76	6.89	7.02	7.15	7.28	7.41	7.54	7.67
6	7.80	7.93	8.06	8.19	8.32	8.45	8.58	8.71	8.84	8.97
7	9.10	9.23	9.36	9.49	9.62	9.75	9.88	10.01	10.14	10.27
8	10.40	10.53	10.66	10.79	10.92	11.05	11.18	11.31	11.44	11.57
9	11.71	11.84	11.97	12.10	12.23	12.36	12.49	12.62	12.75	12.88
10	13.01	13.14	13.27	13.40	13.53	13.66	13.79	13.92	14.05	14.18
11	14.30	14.44	14.57	14.70	14.83	14.95	15.09	15.22	15.35	15.48
12	15.60	15.74	15.87	16.00	16.13	16.26	16.39	16.52	16.65	16.78
13	16.91	17.04	17.17	17.30	17.43	17.56	17.69	17.82	17.95	18.08
14	18.21	18.34	18.47	18.60	18.73	18.86	18.99	19.12	19.25	19.38
15	19.51	19.64	19.77	19.90	20.03	20.16	20.29	20.42	20.55	20.68
16	20.81	20.94	21.07	21.20	21.33	21.46	21.59	21.72	21.85	21.98
17	22.11	22.24	22.37	22.50	22.63	22.76	22.89	23.02	23.15	23.28
18	23.41	23.54	23.67	23.80	23.93	24.06	24.19	24.32	24.45	24.58
19	24.72	24.85	24.98	25.11	25.24	25.37	25.50	25.63	25.76	25.89
20	26.02	26.15	26.28	26.41	26.54	26.67	26.80	26.93	27.06	27.19
21	27.32	27.45	27.58	27.71	27.84	27.97	28.10	28.23	28.36	28.49
22	28.62	28.75	28.88	29.01	29.14	29.27	29.40	29.53	29.66	29.79
23	29.92	30.05	30.18	30.31	30.44	30.57	30.70	30.83	30.96	31.09
24	31.22	31.35	31.48	31.61	31.74	31.87	32.00	32,13	32.26	32.39
25	32.52	32.65	32.78	32.91	33.04	33.17	33.30	33.43	33.56	33.69
26	33.82	33.95	34.08	34.21	34.34	34.47	34.60	34.73	34.86	34.99
27	35.12	35.25	35.38	35.51	35.64	35.77	35.90	36.03	36.16	36.29
28	36.42	36.55	36.68	36.81	36.94	37.07	37.20	37.33	37.46	37.59
29	37.73	37.86	37.99	38.12	38.25	38.38	38.51	38.64	38.77	38.90
30	39.03	39.16	39.29	39.42	39.55	39.68	39.81	39.94	40.07	40.20

Urea. Table for a Temperature of 15° C.

	1	1/10	2/10	3/10	4/10	5/10	6/10	7/10	8/10	9/10
1	1.28	1.41	1.53	1.66	1.79	1.92	2.04	2.17	2.30	2.43
2	2.56	2.69	2.81	2.94	3.07	3.20	3.33	3.46	3.58	3.71
3	3.84	3.97	4.10	4.22	4.35	4.48	4.61	4.74	4.87	4.99
4	5.12	5.25	5.38	5.50	5.63	5.76	5.89	6.02	6.14	6.27
5	6.40	6.53	6.60	6.79	6.91	7.04	7.17	7.30	7.43	7.55
6	7.68	7.81	7.94	8.07	8.19	8.32	8.45	8.58	8.71	8.83
7	8.96	9.09	9.22	9.35	9.48	9.60	9.73	9.86	9.99	10.12
8	10.24	10.37	10.50	10.63	10.76	10.88	11.01	11.14	11.27	11.40
9	11.53	11.65	11.78	11.91	12.04	12.17	12.29	12.42	12.55	12.68
10	12.81	12.93	13.06	13.19	13.32	13.45	13.57	13.70	13.83	13.96
11	14.09	14.22	14.34	14.47	14.60	14.73	14.86	14.98	15,11	15.24
12	15.37	15.50	15.62	15.75	15.88	16.01	16.14	16.26	16.39	16.52
13	16.65	16.78	16.91	17.03	17.16	17.29	17.42	17.55	17.67	17.80
14	17.93	18.06	18.19	18.31	18.44	18.57	18.70	18.83	18.95	19.08
15	19.21	19.34	19.47	19.60	19.72	19.85	19.98	20.11	20.24	20.36
16	20.49	20.62	20.75	20.88	21.00	21.13	21.26	21.39	21.52	21.64
17	21.77	21.90	22.03	22.16	22.29	22.41	22.54	22.67	22.80	22.93
18	23.05	23.18	23.31	23.44	23.57	23.69	23.82	23.95	24.08	24.21
19	24.34	24.46	24.59	24.72	24.85	24.98	25.10	25.23	25.36	25.49
20	25.62	25.74	25.87	26.00	26.13	26.26	26.38	26.51	26.64	26.77
21	26.90	27.03	27.15	27.28	27.41	27.54	27.67	27.79	27.92	28.05
22	28.18	28.31	28.43	28.56	28.69	28.82	28.95	29.07	29.20	29.33
23	29.46	29.59	29.72	29.84	29.97	30.10	30.23	30.36	30.48	30.61
24	30.74	30.87	31.00	31.12	31.25	31.38	31.51	31.64	31.76	31.89
25	32.02	32.15	32.28	32.41	32.53	32.66	32.79	32.92	33.05	33.17
26	33.30	33.43	33.56	33.69	33.81	33.94	34.07	34.20	34.33	34.45
27	34.58	34.71	34.84	34.97	35.10	35.42	35.35	35.48	35.61	35.74
28	35.86	35.99	36.12	36.25	36.38	36.50	36.63	36.76	36.89	37.02
29	37.15	37.27	37.40	37.53	37.66	37.79	37.91	38.04	38.17	38.30
30	38.43	38.55	38.68	38.81	38.94	39.07	39.12	39.32	39.45	39.58

Urea. Table for a Temperature of 20° C.

	1	1/10	2/10	3/10	4/10	5/10	6/10	7/10	8/10	9/10
1	1.26	1.38	1.51	1.63	1.76	1.89	2.01	2.14	2.26	2.39
2	2.52	2.64	2.77	2.90	3.02	3.16	3.27	3.40	3.53	3.65
3	3.78	3.91	4.03	4.16	4.28	4.41	4.54	4.66	4.79	4.91
4	5.04	5.17	5.29	5.42	5.54	5.67	5.80	5.92	6.05	6.17
5	6.30	6.43	6.55	6.68	6.81	6.93	7.06	7.18	7.31	7.44
6	7.56	7.69	7.81	7.94	8.07	8.19	8.32	8.44	8.57	8.70
7	8.82	8.95	9.08	9.20	9.33	9.45	9.58	9.71	9.83	9.96
8	10.08	10.21	10.34	10.46	10.59	10.71	10.84	10.97	11.09	11.22
9	11.35	11.47	11.60	11.72	11.85	11.98	12.10	12.23	12.35	12.48
10	12.61	12.73	12.86	12.98	13.11	13.24	13.36	13.49	13.61	13.74
11	13.87	13.99	14.12	14.25	14.37	14.50	14.62	14.75	14.88	15.00
12	15.13	15.25	15.38	15.51	15.63	15.76	15.88	16.01	16.14	16.26
13	16.39	16.52	16.64	16.77	16.89	17.02	17.15	17.27	17.40	17.52
14	17.65	17.78	17.90	18.03	18.15	18.28	18.41	18.53	18.66	18.78
15	18.91	19.04	19.16	19.29	19.42	19.54	19.67	19.79	19.92	20.05
16	20.17	20.30	20.42	20.55	20.68	20.80	20.93	21.05	21.18	21.31
17	21.43	21.56	21.69	21.81	21.94	22.06	22.19	22.32	22.44	22.57
18	22.69	22.82	22.95	23.07	23.20	23.32	23.45	23.53	23.70	23.83
19	23.96	24.08	24.21	24.33	24.46	24.59	24.71	24.84	24.96	25.09
20	25.22	25.34	25.47	25.59	25.72	25.85	25.97	26.10	26.22	26.35
21	26.48	26.60	26.73	26.86	26.98	27.11	27.23	27.36	27.49	27.61
22	27.74	27.86	27.99	28.12	28.24	28.37	28.49	28.62	28.75	28.87
23	29.00	29.13	29.25	29.38	29.50	29.63	29.76	29.88	30.01	30.13
24	20.26	30.39	30.51	30.64	30.76	30.89	31.02	31.14	31.27	31.39
25	31.52	31.65	31.77	31.90	32.03	32.15	32.28	32.40	32.53	32.66
26	32.78	32.91	33.03	33.16	33.29	33.41	33.54	33.66	33.79	33.92
27	34.04	34.17	34.30	34.42	34.55	34.67	34.80	34.93	35.05	35.18
28	35.30	35.43	35.56	35.68	35.81	35.93	36.06	36.19	36.31	36.44
29	36.57	36.69	36.82	36.94	37.07	37.20	37.32	37.45	37.57	37.70
30	37.83	37.95	38.08	38.20	38.33	38.46	38.58	38.71	38.83	38.96

Urea. Table for a Temperature of 25° C.

	0	$\frac{1}{10}$	$\frac{2}{10}$	$\frac{3}{10}$	$\frac{4}{10}$	$\frac{5}{10}$	$\frac{6}{10}$	$\frac{7}{10}$	$\frac{8}{10}$	$\frac{9}{10}$
1	1.24	1.36	1.49	1.61	1.73	1.86	1.98	2.11	2.23	2.35
2	2.48	2.60	2.73	2.85	2.97	3.10	3.22	3.35	3.47	3.59
3	3.72	3.84	3.97	4.09	4.22	4.34	4.46	4.59	4.71	4.84
4	4.96	5.08	5.21	5.33	5.46	5.58	5.70	5.83	5.95	6.08
5	6.20	6.33	6.45	6.57	6.70	6.82	6.95	7.07	7.19	7.32
6	7.44	7.57	7.69	7.81	7.94	8.06	8.19	8.31	8.43	8.50
7	8.68	8.81	8.93	9.06	9.18	9.30	9.43	9.55	9.68	9.80
8	9.92	10.05	10.17	10.30	10.42	10.54	10.67	10.79	10.92	10.04
9	11.17	11.29	11.41	11.54	11.66	11.79	11.91	12.03	12.16	12.28
10	12.41	12.53	12.65	12.78	12.90	13.03	13.15	13.27	13.40	13.52
11	13.65	13.77	13.89	14.02	14.14	14.27	14.39	14.52	14.64	14.76
12	14.89	15.01	15.14	15.26	15.38	15.51	15.63	15.76	15.88	16.00
13	16.13	16.25	16.38	16.50	16.63	16.75	16.87	17.00	17.12	17.26
14	17.37	17.49	17.62	17.74	17.87	17.99	18.11	18.24	18.36	18.49
15	18.61	18.74	18.86	18.98	19.11	19.23	19.36	19.48	19.60	19.73
16	19.85	19.98	20.10	20.22	20.35	20.47	20.60	20.72	20.84	20.97
17	21.09	21.22	21.34	21.47	21.59	21.71	21.84	21.96	22.09	22.21
18	22.33	22.46	22.58	22.71	22.83	22.95	23.08	23.20	23.33	23.45
19	23.58	23.70	23.82	23.95	24.07	24.20	24.32	24.44	24.57	24.69
20	24.82	24.94	25.06	25.19	25.31	25.44	25.56	25.68	25.81	25.93
21	26.06	26.18	26.30	26.43	26.55	26.68	26.80	26.92	27.05	27.17
22	27.30	27.42	27.55	27.67	27.79	27.92	28.04	28.17	28.29	28.41
23	28.54	28.66	28.79	28.91	29.04	29.16	29.28	29.41	29.53	29.66
24	29.78	29.90	30.03	30.15	30.28	30.40	30.52	30.65	30.77	30.90
25	31.02	31.15	31.27	31.39	31.52	31.64	31.77	31.89	32.01	32.14
26	32.26	32.39	32.51	32.63	32.76	32.88	33.01	33.13	33.25	33.38
27	33.50	33.63	33.75	33.88	34.00	34.12	34.25	34.37	34.50	34.62
28	34.74	34.87	34.99	35.12	35.24	35.36	35.49	35.61	35.74	35.86
29	35.99	36.11	36.23	36.36	36.48	36.61	36.73	36.85	36.98	37.10
30	37.23	37.35	37.47	37.60	37.72	37.85	37.97	38.09	38.22	38.24

Urea. Table for a Temperature of 30° C.

	0	$\frac{1}{10}$	$\frac{2}{10}$	$\frac{3}{10}$	$\frac{4}{10}$	$\frac{5}{10}$	$\frac{6}{10}$	$\frac{7}{10}$	$\frac{8}{10}$	$\frac{9}{10}$
1	1.22	1.34	1.46	1.58	1.71	1.83	1.95	2.07	2.19	2.32
2	2.44	2.56	2.68	2.80	2.93	3.05	3.17	3.29	3.41	2.54
3	3.66	3.78	3.90	4.03	4.15	4.77	4.39	4.51	4.64	4.76
4	4.88	5.00	5.12	5.25	5.37	5.49	5.61	5.73	5.86	5.98
5	6.10	6.22	6.35	6.47	6.59	6.71	6.83	6.96	7.08	7.20
6	7.32	7.44	7.57	7.69	7.81	7.93	8.05	8.18	8.30	8.42
7	8.54	8.67	8.79	8.91	9.03	9.15	9.28	9.40	9.52	9.64
8	9.76	9.89	10.01	10.13	10.25	10.37	10.50	10.62	10.74	10.86
9	10.99	11.11	11.23	11.35	11.47	11.60	11.72	11.84	11.96	12.08
10	12.21	12.33	12.45	12.57	12.69	12.82	12.94	12.06	13.18	13.30
11	13.43	13.55	13.67	13.79	13.92	14.04	14.16	14.28	14.40	14.53
12	14.65	14.77	14.89	15.01	15.14	15.26	15.38	15.50	15.62	15.75
13	15.87	15.99	16.11	16.24	16.36	16.48	16.60	16.72	16.85	16.97
14	17.09	17.21	17.33	17.46	17.58	17.70	17.82	17.94	18.07	18.19
15	18.31	18.43	18.56	18.68	18.80	18.92	19.04	19.17	19.29	19.41
16	19.53	19.65	19.78	19.90	20.02	20.14	20.26	20.39	20.51	20.63
17	20.75	20.88	21.00	21.12	21.24	21.36	21.49	21.61	21.73	21.85
18	21.97	22.10	22.22	22.34	22.46	22.58	22.71	22.83	22.95	23.07
19	23.19	23.32	23.44	23.56	23.68	23.81	23.93	24.05	24.17	24.29
20	24.42	24.54	24.66	24.78	24.90	25.03	25.15	25.27	25.39	25.51
21	25.65	25.76	25.88	26.00	26.13	26.25	26.37	26.49	26.61	26.74
22	26.86	26.98	27.10	27.22	27.35	27.47	27.59	27.71	27.83	27.96
23	28.08	28.20	28.32	28.45	28.57	28.69	28.81	28.93	29.06	29.18
24	29.30	29.42	29.54	29.67	29.79	29.91	30.03	30.15	30.28	30.40
25	30.52	30.64	30.77	30.89	31.01	31.13	31.25	31.38	31.50	31.62
26	31.74	31.86	31.99	32.11	32.23	32.35	32.47	32.60	32.72	32.84
27	32.96	33.09	33.21	33.33	33.45	33.57	33.70	33.82	33.94	34.06
28	34.18	34.31	34.43	34.55	34.67	34.79	34.92	35.04	35.16	35.28
29	35.41	35.53	35.65	35.77	35.89	36.02	36.14	36.26	36.38	36.50
30	36.63	36.75	36.87	36.99	37.11	37.24	37.36	37.48	37.60	37.72

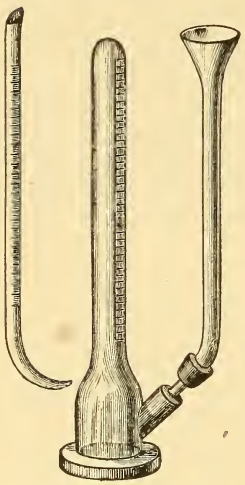
directly the number of grammes or grains of urea contained in the amount of urine employed.[1]

Green's apparatus (Fig. 98) consists of a tube, graduated in cubic centimeters, which is blown out at the bottom into a wider portion, and holds in all about 50 to 60 c.c. The bulb is provided with a side-tube, into which a bent funnel-tube can be inserted for the purpose of equalizing the pressure. The side-tube having been detached, the apparatus is filled with sodium hypobromite solution, when 2 c.c. of urine (diluted if necessary) are introduced by means of a graduated and bent pipette. After all bubbles of gas have disappeared the funnel-tube is inserted into the side-opening and filled with hypobromite solution

Fig. 100.

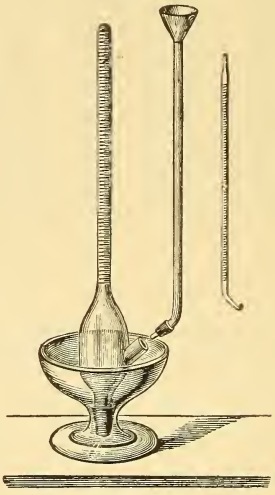
Hüffner's ureometer.

Fig. 98.



Green's ureometer.

Fig. 99.



Marshall's ureometer.

until the level in both tubes is the same. The volume is then noted, corrected, and the corresponding amount of urea calculated as described.

Marshall's apparatus is a conveniently modified form of Green's, and is used in the same manner (Fig. 99).

Hüffner's apparatus is excellent (Fig. 100). It consists of a small bulb, A, of 5 c.c. capacity, which is separated from a larger bulb, C, holding about 100 c.c., by a well-oiled glass stopcock. The upper end of C is drawn out to such an extent that the eudiometer D, which is about 30 cm. long, 2 cm. wide, and divided into fifths

[1] Instead of employing the solution described on page 355, it is sufficient to fill the long arm of the tube with a 40 per cent. solution of caustic soda, and to add 1 c.c. of bromine and a sufficient amount of water to fill the bend of the tube.

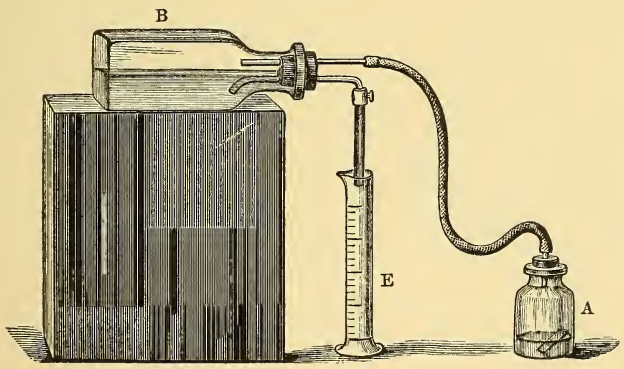
of a cubic centimeter, can be passed over it for a short distance. The bowl E, fitted over C by means of a cork, serves to hold a portion of the hypobromite solution.

The exact capacity of A and of the lumen of the stopcock must be separately determined for each instrument.

Method.—The bulb A and the lumen of the stopcock are filled with urine (which has been diluted, if necessary). The stopcock having been closed, C is washed out carefully with distilled water and filled with the hypobromite solution until the liquid in the dish stands several centimeters above the mouth of C. The eudiometer is next filled with the same solution, carefully submerged in the liquid contained in the dish, and adjusted over the mouth of C. The urine in A is then allowed to mix with the hypobromite solution very gradually, by opening the stopcock. After all bubbles of gas have disappeared the eudiometer is transferred to a cylinder filled with water and thoroughly immersed. After twenty to thirty minutes the level of the liquid in the tube and that of the outside water are equalized and the reading taken. The temperature of the water being likewise noted, the volume of the gas is corrected and the corresponding amount of urea calculated.

Squibb's Method.—This method, like that of Doremus, may be highly recommended to the practitioner for its simplicity. The apparatus (Fig. 101) consists of two ordinary medicine-bottles, A and

FIG. 101.



Squibb's ureometer.

B. In A the nitrogen is evolved. B is closed by a doubly perforated rubber stopper, a straight tube passing through the upper aperture and connecting with the bottle A. Another tube, bent downward and carrying a clamp, as seen in the figure, leads to a graduated cylinder, E. B contains a sufficient amount of water for the bent tube to dip into; 25 to 30 c.c. of the hypobromite solution and a

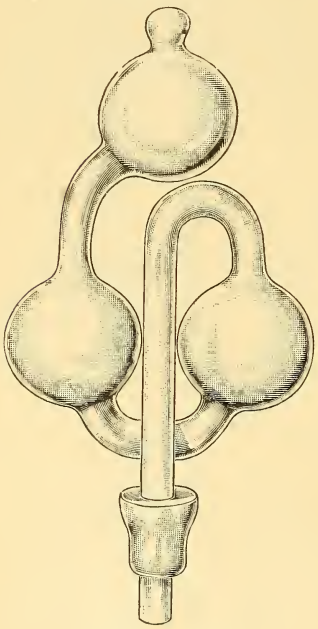
small tube containing 2 c.c. of urine (diluted if necessary, according to the specific gravity) are placed in A, the clamp at E being closed. The rubber stopper is now firmly inserted and E opened, when a few drops of water, which may be disregarded, will escape. The graduated cylinder is then placed beneath the outflow-tube and the bottle A inclined. The nitrogen collecting in B displaces its own volume of water, which flows out and is collected in E, whence the corresponding amount of urea may be calculated or read off from the accompanying tables (pages 429–431).

It should be mentioned that sodium hypobromite liberates nitrogen not only from urea, but also from the other nitrogenous constituents of the urine; the error thus incurred, however, appears just to counterbalance the deficit in the amount of nitrogen obtained, and corresponds to 1 gramme of urea.

If greater accuracy is required, the method recently suggested by Folin may be employed.[1]

Method of Folin.—This is based upon the following considerations: At a temperature of about 160° C. crystallized magnesium chloride, $MgCl_2.6H_2O$, boils in its water of crystallization. In such a solution urea is quantitatively decomposed into ammonia and carbon dioxide within one-half hour. If the process is carried out in acid solution, the ammonia can subsequently be distilled off after rendering the mixture alkaline, and is then titrated. The corresponding amount of urea is ascertained by calculation. At the same time, however, the preformed ammonia is obtained, and it is hence necessary to eliminate this source of error by a separate estimation of this form. This is conveniently done according to the method which has likewise been suggested by Folin (see below).

Fig. 102.



Folin's safety-tube.

Method.—Three c.c. of urine carefully measured with a 5 c.c. pipette graduated in twentieths are placed in an Erlenmeyer flask of 200 c.c. capacity, together with 20 grammes of magnesium chloride and 2 c.c. of concentrated hydrochloric acid. (The magnesium chloride usually contains a small amount of ammonia, which must be separately determined.) The flask is closed

[1] O. Folin, Zeit. f. physiol. Chem., vol. xxii. p. 504, and vol. xxxvi. p. 333.

with a perforated stopper through which a specially constructed safety-tube passes (see Fig. 102).[1] The mixture is now boiled until the drops flowing back through the tube produce a hissing sound on coming in contact with the solution. After this point has been reached, the boiling is continued more moderately for about forty-five minutes. Immoderate foaming during this process and the subsequent distillation is guarded against by adding a small piece of paraffin (about the size of 2 coffee beans). The solution while still hot is carefully diluted to about 500 c.c.—at first by allowing the water to flow drop by drop through the tube; it is then transferred to a 1000 c.c. retort, treated with about 7 or 8 c.c. of a 20 per cent. solution of sodium hydrate, and the ammonia distilled off into a measured amount of a decinormal solution of sulphuric acid. The distillation may be interrupted when about 350 c.c. have passed over (viz., after about sixty minutes). The distillate is boiled for a moment to remove any carbon dioxide which may be present in solution, and on cooling is titrated to determine the excess of acid. Each cubic centimeter of the decinormal ammonia present in the distillate corresponds to 0.003 gramme, viz., to 0.1 per cent. of urea.

From this result the amount of preformed ammonia and that present in the 20 grammes of magnesium chloride must be deducted.

Estimation of Nitrogen.—For the purpose of estimating the total amount of nitrogen in the urine, the method of Kjeldahl or that of Will-Varrentrapp is most conveniently employed.

Kjeldahl's Method.[1]—*Principle.*—The organic matter of the urine is decomposed by means of sulphuric acid, when all the nitrogen which is not present in combination with oxygen is transformed into ammonia. After adding sodium hydrate in excess the ammonia is then distilled off and received in a known quantity of titrated acid, the excess being retitrated with sodium hydrate. In this manner the amount of ammonia and the corresponding quantity of nitrogen are ascertained, it being remembered that 17 grammes of ammonia correspond to 14 grammes of nitrogen.

Reagents required:

1. Gunning's mixture. This consists of 15 c.c. of concentrated sulphuric acid, 10 grammes of potassium sulphate, and 0.5 gramme of cupric sulphate.
2. A solution of sodium hydrate containing 270 grammes in the liter (sp. gr. 1.243).
3. Pulverized talcum or granulated zinc.
4. A one-fourth normal solution of sulphuric acid.
5. A one-fourth normal solution of sodium hydrate.

Apparatus required (see Fig. 103): This consists of a retort of

[1] The tube can be obtained from Messrs. Eimer & Amend, of New York.

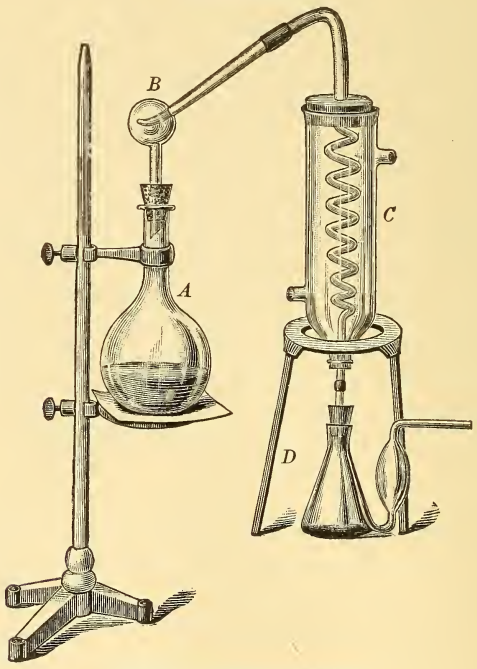
[2] J. Kjeldahl, "Neue Methode zur Bestimmung des Stickstoffes in organischen Körpern," Zeit. f. analyt. Chem., 1883, vol. xxii. p. 366.

about 750 c.c. capacity (*A*), which is connected with a Kjeldahl distilling tube (*B*), and through this with a Städeler condenser (*C*). The ammonia is received in the nitrogen bulb at *D*. In addition a Kjeldahl digesting flask of 200 to 300 c.c. capacity is required.

METHOD.—Five or 10 c.c. of urine are placed in the digesting flask and treated with Gunning's mixture. To this end, it is best to add the sulphuric acid and cupric sulphate first, to heat until sulphuric acid vapors are given off in abundance, and then to add the potassium sulphate. The heating is continued until the solution becomes entirely clear and almost colorless, the flask being inclined at an angle of about 45 degrees. *Vigorous ebullition should be avoided.*

Upon cooling, the contents of the flask are transferred to the retort with the aid of a little water, and slowly treated with a moderate excess of the sodium hydrate solution. As a general rule, 40

FIG. 103.

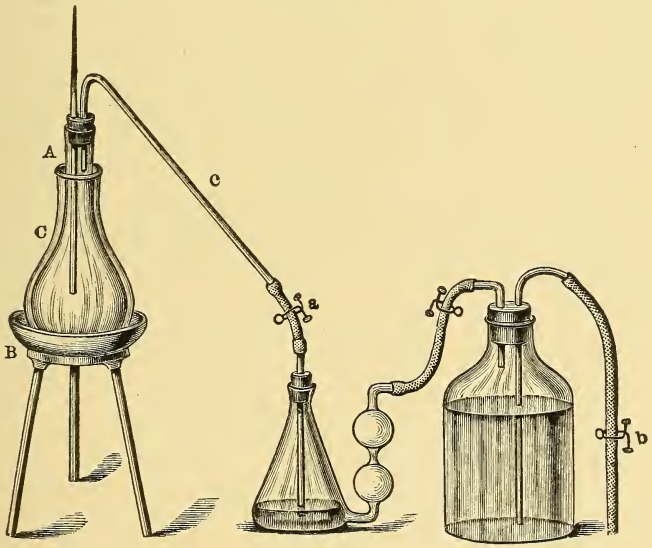


Kjeldahl's nitrogen apparatus.

c.c. for each 5 c.c. of sulphuric acid are sufficient. A little pulverized talcum or a few pieces of granulated zinc are finally added; the retort is connected with the condenser, and the distillation begun. This is continued until about two-thirds of the solution have passed over. The distillate is received in the nitrogen bulb, which should

contain a carefully measured quantity of the one-fourth normal solution of sulphuric acid. As a general rule, 30 c.c. are sufficient. As soon as the distillation is completed the condenser is disconnected, washed out with a small amount of distilled water, and the washings added to the distillate. After the addition of a few drops of tincture of cochineal or dimethyl-amido-azo-benzol the excess of sulphuric acid is retitrated with the one-fourth normal solution of sodium hydrate, and the amount found deducted from the 30 c.c. used. The titration should be continued until every trace of yellow has disappeared and a pure rose color is obtained, or, in the case of the dimethyl-amido-azo-benzol, until the last trace of red has disappeared and the solution has turned yellow. The difference multiplied by 0.0035 will then indicate the amount of nitrogen present in the 5 or 10 c.c of urine. The corresponding amount of urea is found by multiplying this figure by 20.

Fig. 104.



Apparatus for the determination of nitrogen.

As Kjeldahl's method presupposes a thorough knowledge of chemical technique, it is well to make at least two parallel estimations in every case.

Will-Varrentrapp's Method (as modified by Seegen-Schneider).[1]—*Principle.*—If nitrogenous organic material is heated in intimate contact with soda-lime, all the nitrogen is given off in the form of ammonia, which is received in a known quantity of acid; the excess,

[1] Will-Varrentrapp, see Leube-Salkowski, Die Lehre vom Harn.

not used in the neutralization of the ammonia, is then determined by titration with a solution of sodium hydrate of known strength. The amount held by the ammonia is thus ascertained, and from it the corresponding amount of nitrogen, it being remembered that 17 grammes of ammonia correspond to 14 grammes of nitrogen.

Reagents required:

1. A quantity of thoroughly fused soda-lime, which, while still hot, should be placed in a well-stoppered bottle, where it may be kept ready for use for a long time.
2. A normal solution of sulphuric acid.
3. A normal solution of sodium hydrate.

Apparatus required: As is apparent from the accompanying diagram (Fig. 104), the apparatus consists of a Kjeldahl digesting flask, *A*, of about 100 c.c. capacity, and provided with a neck 10 to 12 cm. long; this is placed in a copper crucet, *B*, and imbedded in sand. The crucet is placed upon a pipe-stem triangle over the flame. The neck of the flask is surrounded by a hood of copper or tin plate, *C*, moulded to the flask and reaching not higher than 1.5 cm. below the rubber stopper. The latter is doubly perforated, a tube, *e*, drawn out to a point and closed at the free end, passing through one aperture and extending about half-way down the flask, while the second passes through the other opening. This second tube, *c*, is connected by means of a short piece of rubber tubing, upon which a clamp is placed, with a Will-Varrentrapp apparatus. The latter is connected by rubber tubing, upon which a clamp is likewise placed, with an aspirating-bottle filled with water and provided with a siphon tube.

Method.—Ten c.c. of the normal sulphuric acid solution are placed in the Will-Varrentrapp apparatus, together with a few cubic centimeters of a 1 per cent. alcoholic solution of phenolphthalein. A layer of sand about 1 cm. in height is placed in the crucet, the clamp *a* closed, and the flask filled to about one-half its height with the soda-lime, when the hood is adjusted and 5 c.c. of urine are allowed to flow upon the soda. The rubber stopper is quickly adjusted, the rubber tube having been previously connected with the Will-Varrentrapp apparatus. The clamp *a* is now opened, the crucet filled with sand, and the heating begun. This is at first done carefully with a small flame, but increased gradually until a full heat is applied. This is continued for one-half to three-quarters of an hour. When drops of moisture are no longer visible in the tube *c*, or when the evolution of gas has entirely ceased, the rubber tube of the aspirating-bottle *d* is slipped on to the Will-Varrentrapp apparatus, the clamp *b* slightly opened, the tip of *e* broken off, and air allowed to pass slowly through the entire system for a quarter of an hour, when the flame is extinguished. The Will-Varrentrapp apparatus is then detached and its contents titrated with the normal solution of sodium hydrate.

The number of cubic centimeters of the sodium hydrate solution employed is deducted from 10 (the number of cubic centimeters of the normal sulphuric acid solution, 1 c.c. of the latter being equivalent to 1 c.c. of the former), the difference giving the number of cubic centimeters of the normal sulphuric acid solution neutralized by the ammonia evolved from 5 c.c. of urine. This number multiplied by 20 will then represent the number of cubic centimeters required to neutralize the ammonia contained in 100 c.c. of urine. As 1000 c.c. of the normal solution of sulphuric acid correspond to 17 grammes of ammonia, or 14 grammes of nitrogen, the number of cubic centimeters of the sulphuric acid solution corresponding to 100 c.c. of urine will be found from the equation : $1000 : 14 :: x : y$; and $y = 0.014\,x$, in which x represents the number of cubic centimeters required to neutralize the amount of ammonia evolved from 100 c.c. of urine, and y the corresponding amount of nitrogen—*i. e.*, the percentage of nitrogen.

If the nitrogen is to be calculated in terms of urea, this is done according to the equation : $1000 : 30\ (= 14\text{N}) :: x : y$; and $y = 0.03\,x$ = percentage of urea, in which x represents, as above, the number of cubic centimeters of sulphuric acid neutralized by the ammonia, viz., nitrogen, contained in 100 c.c. of urine, and y the urea corresponding to this amount.

Ammonia.

Every urine contains a small amount of ammonia, which normally varies but little, and corresponds to from 4.1 to 4.64 per cent. of the total amount of nitrogen, viz., to about 0.7 gramme in the twenty-four hours. It is present in combination with the various acids of the urine, and in all likelihood represents a small amount of the ammonia which has not been transformed into urea, but has been utilized to saturate the affinities of a slight excess of acid, formed during the nitrogenous metabolism of the body, over the available fixed alkalies. In this manner indeed the body is capable of guarding against the appearance of free acid in the blood, and it is for this reason, as I have already pointed out, that free acid cannot occur in the urine. This safeguard, however, does not exist in the herbivorous animals, in which the fixed alkali only is apparently available for the neutralization of acids, and we consequently find that whereas in dogs, for example, an acid intoxication occurs only after the administration of very large quantities of acid, the herbivora rapidly succumb after the ingestion of comparatively small amounts.

In man an increased elimination of ammonia is observed whenever an increased formation of acids occurs, or whenever a sufficient supply of oxygen is not available. In the latter case, no doubt, the increased elimination is owing to the fact that in consequence of the deficient supply of oxygen the synthetic formation of urea

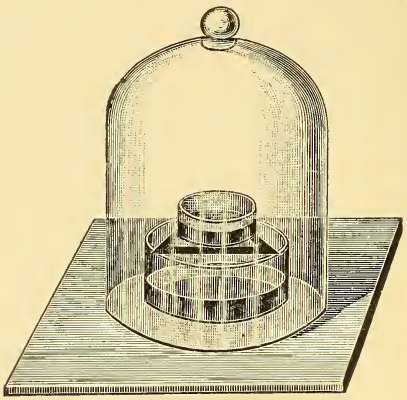
from ammonium lactate is impeded in the liver. As this organ, moreover, is the principal seat of the synthesis of urea, we can readily understand that extensive parenchymatous degeneration, as in acute yellow atrophy, in phosphorus poisoning, etc., will lead to an increased elimination of ammonia.

In any event, the relative increase of the ammonia is the essential factor, while variations in its absolute quantity are of secondary importance. Some of the results which have been obtained in various diseases are given in the following table:

	Per cent.
Normal values	4.10– 4.64
Febrile diseases	5.72– 6.70
Carcinoma of the liver	6.40–24.50
Liver abscess (actinomycosis)	10.60
Circulatory dyspnœa	13.10–32.20
Respiratory dyspnœa	6.60–14.30

Abnormally high absolute values are quite constantly observed in diabetes, in which an elimination of from 4 to 5 grammes may be regarded as common. In one instance 5.94 grammes were excreted in twenty-four hours. In a general way the amount of ammonia in cases of diabetes gives an idea of the amount of organic acids; but, as Herter has pointed out, we cannot detect moderate quantities of organic acids in this way (see Oxybutyric Acid).

Fig. 105.



Desiccator.

Very curiously, diminished elimination of ammonia is observed in many cases of nephritis so long as symptoms of venous stasis do not exist.

In a case of pernicious anæmia relative amounts, varying between 3.3 and 5.6 per cent., were obtained during the days immediately preceding death.

Quantitative Estimation.—Schlösing's Method.—*Principle.*—A carefully measured amount of urine is treated with milk of lime and placed under a bell, together with a vessel containing a known amount of a normal solution of sulphuric acid. In the course of time the ammonia is liberated and absorbed by the acid. This is then titrated, and the deficit expressed in terms of ammonia.

METHOD.—Twenty-five c.c. of perfectly fresh, filtered urine are placed in a flat dish, upon the plate of a desiccator, as shown in Fig. 105. Above this is a smaller dish containing 10 c.c. of a normal solution of sulphuric acid. The urine is treated with 10 c.c. of milk of lime, the bell is carefully adjusted after lubrication with tallow, and the apparatus allowed to stand for at least three or four days. The excess of acid remaining is then titrated with a one-fourth normal solution of sodium hydrate, using as an indicator a few drops of a saturated aqueous solution of methyl-orange until the red color has turned to yellow. To neutralize the 10 c.c. of the acid, 40 c.c. of the one-fourth normal solution are required. The difference is referable to the partial neutralization by the ammonia, and is expressed in milligrammes. One c.c. of the one-fourth normal solution corresponds to 4.25 mgrms. of ammonia.

Precautions: 1. In every case the urine must be perfectly fresh. Decomposition is best guarded against during its collection by adding about 10 to 20 c.c. of chloroform to the portion first voided.

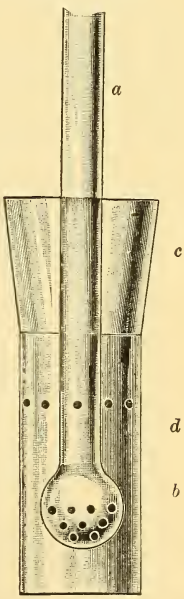
2. Urines which are undergoing ammoniacal decomposition should not be utilized for examination.

3. Concentrated or albuminous urines must be kept under the bell for from five to eight days, new portions of acid being used when in doubt as to the complete liberation of the ammonia.

Owing to a slight deposition of moisture on the inner surface of the bell and a consequent retention of traces of ammonia in this form, the resulting figures are too low. The error thus incurred, however, is insignificant.

More satisfactory than this older method is the following method, which has been suggested by Folin:

FIG. 106.



Absorption-tube.

Folin's Method.—Ten c.c. of urine are diluted to about 450 c.c., treated with a small amount of burnt magnesia (0.5 gramme), and boiled for forty-five minutes, the distillate being received in decinormal sulphuric acid through an absorption-tube, such as the one pictured in Fig. 106. This consists of a glass tube, *a*, measuring about 8 mm. in diameter, one extremity of which has been blown into the small bulb *b*. By means

of a heated platinum wire five or six holes, each about 1 mm. in diameter, are made in the bulb; *c* is a rubber stopper which fits into the second tube *d*. This is merely a test-tube (2.5 cm. in diameter) which has been cut about 7.5 cm. from the upper end. About 3 cm. from the upper margin this tube is provided with six or seven holes as in the bulb *b*. The entire apparatus is directly immersed in the decinormal acid and insures the complete absorption of the ammonia in one flask, even if this contains only 5–10 c.c. of the acid. The ammonia is then determined by titration as above, using alizarin red as indicator; 2 drops of a 1 per cent. solution suffice for 200–300 c.c. The titration is carried to the red point, not to the violet. As a small amount of urea, however, is decomposed during the prolonged ebullition, it is necessary to ascertain separately the quantity of ammonia which is referable to this source. To this end, the retort is opened at the expiration of forty-five minutes, and an amount of water added which is approximately equivalent to that of the distillate. The distillation is then continued for another period of forty-five minutes; the distillate is received in decinormal sulphuric acid, and the ammonia referable to decomposition of the urea estimated as before. The difference between the two results indicates the amount of preformed ammonia that was originally present.

This method is also applicable for the determination of ammonia in the blood.

LITERATURE.—Hallervorden, Arch. f. exper. Path., vol. xii. p. 237. Stadelmann, Deutsch. med. Woch., 1889, p. 942. Michaelis, Ibid., 1900, p. 276. O. Folin, Zeit. f. physiol. Chem., vol. xxxii. p. 575; and Ibid., 1902, vol. xxxvii. p. 161.

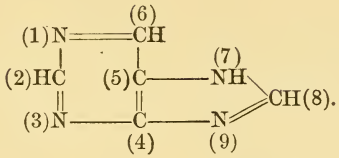
Uric Acid.

According to our present views, uric acid, in man, is not formed during the decomposition of all albuminous substances, as was formerly supposed, but constitutes a specific product of decomposition of one class of albumins only, namely, the nucleins.[1] It appears, moreover, that the mother-substance of uric acid is confined to the nuclear nucleins, viz., to those containing a nucleinic acid radicle; while the paranucleins, in which this is lacking, are without effect upon the elimination of uric acid. According to Kossel,[2] four different forms of nucleinic acid exist, viz., adenylic acid, guanylic acid, sarcylic acid, and xanthylic acid, and the supposition is that each of these contains one base, viz., adenin, guanin, sarcin or hypoxanthin, and xanthin. These basic substances are collectively spoken of as the *xanthin, alloxur*, or *purin bases*. According to Emil Fischer,[1] they are derived from a hypothetical compound which he terms *purin*, and which he supposes to be constituted as shown in the formula.

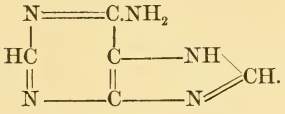
[1] C. E. Simon, Physiological Chemistry, Lea Bros. & Co.

[2] A. Kossel u. A. Neumann, "Ueber Nukleinsäure u. Thyminsäure," Zeit. f. physiol. Chem., vol. xxii. p. 74.

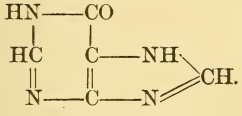
[3] E. Fischer, Ber. d. Deutsch. chem. Ges., 1897, vol. xxx. p. 549.



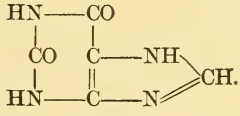
By substituting the group NH_2 for the H atom at 6, adenin thus results, and is hence also spoken of as 6-aminopurin :



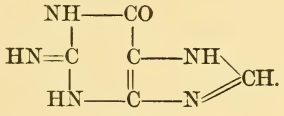
Hypoxanthin, according to this conception, would be 6-oxypurin ; xanthin 2, 6-dioxypurin, and guanin 2-amino-6-oxypurin, as shown by the structural formulæ :



Hypoxanthin.

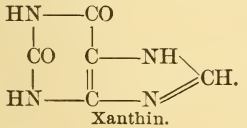


Xanthin.

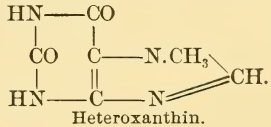


Guanin.

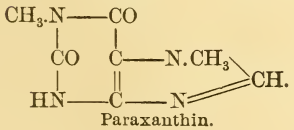
From the structural formula of purin it is also apparent that still other derivatives of this substance may exist, and as a matter of fact others are known, viz., mono-methylxanthin or heteroxanthin, di-methylxanthin or paraxanthin, tri-methylxanthin, the isomeric compounds of paraxanthin, viz., theophyllin and theobromin, and others. Their relation to xanthin is shown in the formulæ :



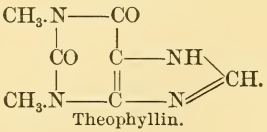
Xanthin.



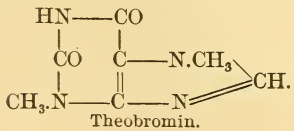
Heteroxanthin.



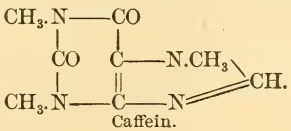
Paraxanthin.



Theophyllin.



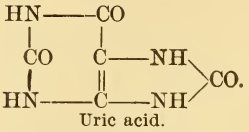
Theobromin.



Caffein.

Two of these bodies, namely, heteroxanthin and paraxanthin, have also been found in urine.

From these basic substances, then, which are found in the nucleinic acid radicle of the nuclear nucleins, uric acid is supposedly derived, and there are numerous facts which go to show that this supposition is in all likelihood correct. It will thus be observed that structurally uric acid is intimately related to the bodies in question, and, like these, contains the purin radicle:



Uric acid.

It may hence be regarded as 2, 6, 8 tri-oxypurin. Uric acid and the xanthin-bases, moreover, qualitatively, all yield the same decomposition-products when treated with fuming hydrochloric acid or hydriotic acid under high pressure; only the quantitative relations vary, as shown in the equations:

$$\underset{\text{Adenin.}}{C_5H_5N_5} + 8H_2O = 4NH_3 + CO_2 + \underset{\text{Glycocoll.}}{CH_2.NH_2.COOH} + \underset{\text{Formic acid.}}{2H.COOH}.$$

$$\underset{\text{Hypoxanthin.}}{C_5H_4N_4O} + 7H_2O = 3NH_3 + CO_2 + CH_2.NH_2.COOH + 2H.COOH.$$

$$\underset{\text{Guanin.}}{C_5H_5N_5O} + 7H_2O = 4NH_3 + 2CO_2 + CH_2.NH_2.COOH + H.COOH.$$

$$\underset{\text{Xanthin.}}{C_5H_4N_4O_2} + 6H_2O = 3NH_3 + 2CO_2 + CH_2.NH_2.COOH + H.COOH.$$

$$\underset{\text{Uric acid.}}{C_5H_4N_4O_3} + 5H_2O = 3NH_3 + 3CO_2 + CH_2.NH_2.COOH.$$

In accordance with this supposed origin of uric acid we find an increased elimination in the urine following the ingestion of all substances which contain purin bases either as such or in the form of nuclear nucleins. At the same time it must be remembered that uric acid may also result from the nucleins of the body-tissues; and we find, as a matter of fact, that during starvation uric acid does not disappear from the urine. The principal source of the uric acid under such conditions are the nucleins of the leucocytes; and according to Horbaczewski[1] and others, this source is indeed more important than the nucleins of the food. According to his idea, the latter call forth an increased elimination of uric acid in only an indirect manner—*i. e.*, by stimulating more strongly than other food-stuffs the cell-formation and cell-destruction of the body. However this

[1] J. Horbaczewski, "Beiträge zur Kenntniss der Bildung von Harnsäure," etc., Monatshefte für Chem., 1891, vol. xii. p. 221; and Wien. Sitzungsber., vol. c.

may be, there can be no doubt that the amount of uric acid eliminated in the urine depends, in the first instance, upon the amount of nucleins or purin bases as such which are ingested, and upon the degree of nuclear destruction which takes place in the body. Other factors, however, also enter into consideration. We thus know that the body is capable of transforming a certain amount of uric acid into urea. This fact was pointed out long ago by Frerichs and Wöhler, and has recently again been confirmed. It was found that after the ingestion of large amounts of nucleins only a certain portion of the nuclear nitrogen is eliminated as uric acid, and that this amount is extremely variable. Whether individual peculiarities have any part in determining this amount is unknown, but is not improbable. Oxidation on the part of the body-tissues must also be taken into consideration, and it unquestionably varies not only in different people, but also in the same individual at different times. Then again there is evidence to show that under certain conditions uric acid may be formed synthetically in the body. That this is the usual mode of formation in birds and reptiles has been conclusively shown by Minkowski,[2] who found that after extirpation of the liver in geese the greater portion of the urinary nitrogen was eliminated in the form of ammonia in combination with lactic acid. In the human being very little uric acid is in all likelihood formed in this manner under normal conditions, but the possibility of its occurrence, in disease more particularly, should not be overlooked. As uric acid, moreover, may in part at least be eliminated in the feces, it is clear that the amount which appears in the urine cannot be regarded as an accurate index of the degree of nuclear destruction or of the amount which is formed in the body-tissues. That retention of uric acid can further occur in the body, which may or may not be followed by increased elimination, is likewise undoubted.

According to our present knowledge, uric acid is formed in all the organs of the body, including the bone-marrow, the muscles, the spleen, the liver, the kidneys, etc. Under pathological conditions it may also originate in the joints and tendons.

Under normal conditions the daily elimination of uric acid varies between 0.2 and 1.5 grammes, thus constituting $\frac{1}{20}$ to $\frac{1}{120}$ part of the total urinary nitrogen. It is largely influenced by the character of the diet, the amount of exercise taken, the general health of the individual, etc. After the ingestion of large amounts of food rich in nuclear nucleins, such as thymus gland, liver, kidneys, and brain, a corresponding increase in the amount of uric acid is observed. Generally speaking, animal food causes a greater elimination of uric acid than vegetable food, and it is supposed that this difference is

[1] Minkowski, "Ueber den Einfluss d. Leberextirpation auf den Stoffwechsel," Arch. f. exper. Path. u. Pharmakol., 1886, vol. xxi. p. 41.

essentially due to the presence of the extractives of the meat.[1] Of special interest is the increase in the elimination of uric acid which is observed five hours after the ingestion of a full meal. This increase, according to Horbaczewski,[2] is associated with the disappearance of the digestive leucocytosis and consequent leucolysis.

Some observers have attached much importance to the relation existing between the elimination of uric acid and urea, and are inclined to assume the existence of a special *uric acid diathesis* when this relation continuously exceeds the usual standard of 1 : 50 or 1 : 60. This question is, however, an extremely intricate one, and we are scarcely in a position at the present time to speak definitely of the significance of such variations. On the one hand, there can be no doubt that an unusually high uric acid coefficient may be met with in individuals who are apparently in good health, while in others, in whom larger amounts of uric acid are eliminated than are usual, normal or even subnormal values may be found. The entire question of the uric acid diathesis is in a chaotic condition, and it would perhaps be well to speak of such a diathesis only when a distinct *absolute* increase is *continuously* observed. That numerous symptoms of a neurasthenic type are often seen when the uric acid coefficient is increased, is a matter of daily observation, but it would be premature to regard this symptom as a causative factor of the disease in question.[3] Even in gout it can scarcely be said that uric acid has been proved the *materia peccans*, and our knowledge concerning the etiology of the disease is still as obscure as when Garrod[4] showed that an accumulation of uric acid occurred in the blood of such patients. Hitherto it has been supposed that the deposition of urates in the joints and periosteum of gouty patients is referable to a diminished alkalinity of the blood, and that acute paroxysms result whenever an increase in its alkalinity occurrs, leading to a resorption of the urates previously deposited and a consequent flooding of the system with the material in question. As a matter of fact, a considerable diminution in its excretion is observed immediately preceding the attack, while during the paroxysm and immediately following it a corresponding increase is noted. Numerous investigations, however, have shown that distinct changes in the alkalinity of the blood do not occur in gout, and that an increase in the amount of uric acid in the blood is not only observed in this disease, but in other diseases as well which are not associated with gouty symptoms.

[1] A. Hermann, "Abhängigkeit der Harnsäureausscheidung von Nahrungs- und Genussmitteln," Deutsch. Arch. f. klin. Med., 1888, vol. xliii. p. 273. See also W. Camerer, Zeit. f. Biol., N. F., 1896, vol. xv. p. 140.

[2] Horbaczewski, Harnsäureausscheidung u. Leucocytose, Sitzungsber. d. Wiener Akad. d. Wissensch., 1891, Abth. 3. See also Löwit, Studien z. Physiol. u. Path. d. Blutes, 1892. W. Kühnau, "Das Verhältniss d. Harnsäureausscheidung zur Leucocytose," Zeit. f. klin. Med., vol. xxviii. p. 534. P. F. Richter, "Ueber Harnsäureausscheidung und Leucocytose," Ibid., vol. xxvii. p. 290.

[3] C. E. Simon, Am. Jour. Med. Sci., 1899, p. 139, and N. Y. Med. Jour., 1895, p. 330.

[4] A. B. Garrod, On the Nature and Treatment of Gout, 1847.

The conclusion is hence justifiable that the presence of uric acid in the blood *per se* cannot be offered as an explanation of the occurrence of a gouty attack.[1] Futcher,[2] who has recently observed a number of cases of gout with modern methods, states that he almost invariably found that before the onset of the acute symptoms the uric acid is below and often far below 0.4 gramme. On the second or third day after the beginning of the acute symptoms the uric acid curve steadily rises, reaching 0.8 to 1.9 gramme or even higher values than this. With subsidence of the acute symptoms the curve gradually falls below the lower limit of the normal, and in the interval between the acute attacks the excretion may be only 0.1–0.2 gramme daily. In one very marked chronic case Futcher found no uric acid excretion whatever on certain days during the interval. The phosphoric acid curve runs a course almost parallel to that of the uric acid, which suggests quite strongly that even in gout the uric acid is derived from nucleins, and is not formed synthetically, as might possibly be imagined.

The greatest increase in the elimination of uric acid is observed in leukæmia, in which amounts of 5 grammes and even more may be observed in the twenty-four hours. That the increased elimination in this disease is referable to the enormous increase in the number of the leucocytes and consequent leucolysis can scarcely be doubted. In other diseases which are associated with a high grade of leucocytosis, and especially those in which the disease terminates by crisis or hastened lysis, such as erysipelas and pneumonia, a considerable increase is likewise observed, and is referable to the same origin. This increase is especially marked immediately after crisis has occurred, but it not infrequently precedes this by several hours. In the other febrile diseases an absolute increase is less marked and inconstant.

In diabetes a diminished amount of uric acid is usually found. Cases may be seen, however, in which, associated with a diminution or an entire disappearance of the sugar, a most marked increase occurs, amounting in some cases to 3 grammes in the twenty-four hours. To this condition the term *diabetes alternans* has been applied.

In acute articular rheumatism an increased elimination is observed so long as the temperature remains high, while with approaching convalescence the amount returns to normal, and may even fall below normal. In chronic rheumatism, on the other hand, no constant relations have been observed.

In the ordinary forms of anæmia and chlorosis the amount of uric acid is quite constantly diminished, as also in chronic inter-

[1] B. Laquer, Ueber die Ausscheidungsverhältnisse der Alloxurkörper. Bergmann, 1906. (Full literature.) C. von Noorden, Lehrbuch d. Pathologie d. Stoffwechsels, Berlin, 1893. W. Ebstein, "Die Natur u. Behandlung der Gicht," Verhandl. d. VIII. Congr. f. inn. Med., 1889, p. 133.

[2] T. B. Futcher, "The Occurrence of Gout in the United States," Jour. Am. Med. Assoc., 1902, vol. xxxix. p. 1046.

stitial nephritis, chronic lead poisoning, progressive muscular atrophy, and pseudohypertrophic paralysis.

Properties of Uric Acid.—The close relation existing between uric acid and the xanthin-bases has been already considered. By oxidation uric acid is transformed into urea or into substituted ureas, such as allantoin and alloxan, which latter in turn is closely related to parabanic acid or oxalyl-urea and barbituric acid or malonyl-urea.

$$\underset{\text{Uric acid.}}{C_5H_4N_4O_3} + O + H_2O = \underset{\text{Alloxan.}}{C_4H_2N_2O_4} + CO{<}\underset{\text{Urea.}}{NH_2}{}^{NH_2}$$

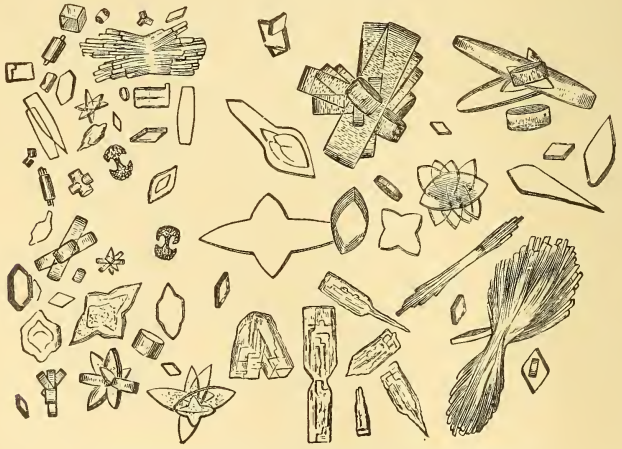
$$\underset{\text{Uric acid.}}{C_5H_4N_4O_3} + H_2O + O = \underset{\text{Allantoin.}}{C_4H_6N_4O_3} + CO_2.$$

Pure uric acid forms a white crystalline powder which is almost insoluble in cold water (1 : 40,000), with difficulty soluble in boiling water (1 : 1800), and insoluble in alcohol and ether. In concentrated sulphuric acid it dissolves with ease, but is precipitated upon dilution with water. In aqueous solutions of the alkaline carbonates and hydrates it dissolves, with the formation of neutral or acid salts, as represented by the equations:

$$C_5H_4N_4O_3 + Na_2CO_3 = C_5H_3NaN_4O_3 + NaHCO_3.$$
$$C_5H_4N_4O_3 + 2Na_2CO_3 = C_5H_2Na_2N_4O_3 + 2NaHCO_3.$$

In freshly voided urine uric acid is said to occur as a quadriurate, viz., as a compound in which one molecule of sodium is in combination with two molecules of uric acid. The quadriurate, however, is readily decomposed with the formation of uric acid and acid urates

FIG. 107.



Various forms of uric acid crystals. (FINLAYSON.)

(biurates). Its solubility in the urine depends upon the amount of water present, the reaction, and the presence of inorganic salts.

When acid sodium phosphate preponderates, the biurate is precipitated, while free uric acid is thrown down when disodic phosphate only is present, and along with this still other acid compounds which are most likely of organic nature. Neutral urates cannot occur in the urine. The basic substances which may occur in the urine in combination with uric acid are sodium, potassium, ammonium, and possibly also calcium and magnesium. These salts may be decomposed by the addition of a sufficiently large quantity of a stronger acid, such as hydrochloric acid, when uric acid is set free. The acid salts are soluble with great difficulty, and are hence precipitated whenever the urine is markedly acid or concentrated, and also when it is exposed to a low temperature. This holds good especially for the acid ammonium compound, and upon this fact Hopkins' quantitative estimation of uric acid is based.

Pure uric acid crystallizes in transparent, colorless, rhombic plates, while that which usually separates from the urine is of a reddish-brown color and may assume a great variety of forms (Fig. 107). Of these, the so-called whetstone-form is the most characteristic (see Sediments). Colorless rhombic platelets may, however, also be seen.

Of the compounds which uric acid forms with the heavy metals, the silver salt is especially important. When a solution of uric acid in ammonia is treated with an ammoniacal solution of silver nitrate (see below) the solution remains clear; but if calcium chloride, sodium chloride, or magnesia mixture is then added, a precipitate forms, which contains the uric acid in combination with silver.

Tests for Uric Acid.—1. **Murexid Test.**—A few crystals are dissolved by means of a few drops of concentrated nitric acid, with the application of heat, upon a porcelain plate, such as the cover of a crucible. The nitric acid is then carefully evaporated, when a yellowish-red spot will remain. Upon cooling, a drop of ammonia is placed upon this spot, when in the presence of uric acid a beautiful purplish-red color develops, owing to the formation of ammonium purpurate (murexid). If now a drop of sodium hydrate solution is added, the color changes to a reddish blue, which disappears upon heating; the reaction thus differs from the somewhat similar xanthin reaction.

2. **Copper Test.**—A few crystals are dissolved in sodium hydrate solution and treated with a few drops of Fehling's solution. Upon the application of heat white copper urate separates out, while red cuprous oxide appears if a relatively large amount of cupric sulphate is present—a point to be remembered in testing for sugar. The reduction of Fehling's solution is due to the formation of allantoin.

3. When treated with sodium hypobromite solution uric acid gives up about 47 per cent. of its nitrogen.

Quantitative Estimation of Uric Acid.—Hopkins' Method.—This method is now commonly used in the clinical laboratory,

and is to be preferred to the more complicated procedures hitherto employed. It is much simpler and equally as accurate as the older methods of Ludwig-Salkowski and of Haycraft. Various modifications of the original method have been suggested.

Principle.—The method is based upon the complete precipitation of uric acid by ammonium salts, and the possibility of accurately titrating the uric acid with potassium permanganate in the presence of sulphuric acid.

Folin's Modification of Hopkins' Method.[1]—To precipitate the uric acid, and also to remove the small amount of mucoid substance which is found in every urine, the following reagent is employed: 500 grammes of ammonium sulphate and 5 grammes of uranium acetate are dissolved in 650 c.c. of water, to which solution 60 c.c. of a 10 per cent. solution of acetic acid are further added. The resulting solution measures about 1000 c.c. Seventy-five c.c. of the reagent are added to 300 c.c. of urine in a flask holding 500 c.c. After standing for five minutes the mixture is filtered through two folded filters, and thus freed from the mucoid body, which is carried down with the uranium phosphate in acid solution. The filtrate is divided into two portions of 125 c.c. each, which are placed in beakers and treated with 5 c.c. of concentrated ammonia. After stirring a little the solutions are set aside until the next day. The supernatant fluid is then carefully poured off through a filter (Schleicher and Schüll, No. 597); the precipitated ammonium urate is collected with the aid of a small amount of a 10 per cent. solution of ammonium sulphate and washed with the same reagent. Traces of chlorides do not interfere with the subsequent titration, and the process of filtration and washing can be completed in from twenty to thirty minutes. The ammonium urate is washed into a beaker, after opening the filter, using about 100 c.c. of water. Fifteen c.c. of concentrated sulphuric acid are then added, and the solution is titrated at once with a one-twentieth normal solution of potassium permanganate. Toward the end of the titration Folin suggests to add the permanganate in portions of two drops at a time, until the *first* trace of a rose color is apparent throughout the entire fluid. Each cubic centimeter of the reagent corresponds to 0.00375 gramme of uric acid. A final correction of 0.003 gramme for each 100 c.c. of urine employed is necessary, owing to the slight extent to which ammonium urate is soluble.

Preparation of the One-twentieth Normal Solution of Potassium Permanganate.—As the molecular weight of potassium permanganate is 157.67, one would expect that a normal solution of the salt should contain this amount in grammes dissolved in 1000 c.c. of water. But the substance generally acts in the presence of free acids, upon deoxidizing substances, by losing 5 atoms of oxygen of the 8 atoms contained in 2 molecules, as is seen in the following equation:

[1] O. Folin u. A. Shaffer, Zeit. f. physiol. Chem., vol. xxxii. p. 552.

$$2KMnO_4 + 5H_2C_2O_4 + 3H_2SO_4 = K_2SO_4 + 2MnSO_4 + 10CO_2 + 8H_2O.$$

It follows that two-fifths of the molecular weight, or 63.068 grammes, are the equivalent of 1 oxygen atom. But as oxygen is diatomic and the volumetric normal is calculated for monatomic values, this number must be divided by 2, and 31.534 grammes of potassium permanganate should therefore be present in 1 liter of normal solution. A one-tenth normal solution would hence contain 3.1534 grammes, and a one-twentieth normal solution 1.576 grammes pro liter. This amount is weighed off and dissolved in 950 c.c. of water, when the solution is brought to the proper degree of dilution (see page 392) by titration with a one-twentieth normal solution of oxalic acid. A one-twentieth normal solution of oxalic acid contains 3.142 grammes of the acid in 1000 c.c. of water. One c.c. of the one-twentieth normal solution of potassium permanganate should correspond to 1 c.c. of the oxalic acid solution. The titration is best conducted by diluting 10 c.c. of the oxalic acid solution to 100 c.c. with distilled water and adding 15 c.c. of concentrated sulphuric acid, so as to bring the temperature of the liquid to from 55° to 65° C. The potassium permanganate solution is then added drop by drop until the red color no longer disappears on stirring, but persists for at least thirty seconds.

Titration with Sodium Hydrate Solution.—This method is not as accurate as the one just described, but suffices for ordinary purposes. The uric acid is precipitated with an ammonium salt, as above. After standing for two hours the ammonium urate is filtered off, washed with a 10 per cent. solution of ammonium sulphate, and brought into a beaker with the aid of a small amount of hot water. The salt is then decomposed by the addition of from 10 to 15 c.c. of a one-tenth normal solution of hydrochloric acid. The mixture is brought to the boiling-point, and the hydrochloric acid not used in the decomposition of the ammonium urate retitrated with a one-tenth normal solution of sodium hydrate, using dimethyl-amido-azo-benzol as an indicator. The amount of hydrochloric acid found is deducted from the 10 or 15 c.c. added, and the result multiplied by 0.0168. The amount of uric acid contained in the original quantity of urine is thus ascertained, to which 0.003 gramme is added for each 100 c.c. of urine used, as above.

Gravimetric Method.—The process is begun as described above. The ammonium urate is decomposed by the addition of from 2 to 3 c.c. of a 25 per cent. solution of hydrochloric acid. This solution is evaporated until crystals of uric acid begin to separate out. These are collected on a dried and weighed filter, and washed successively with water, alcohol (90–95 per cent.), and absolute alcohol, and finally with ether. The mother-liquor and water used in washing are carefully measured, and 0.0004 gramme added to the final result for each 10 c.c.

Haycraft's Method.[1]—This method is based upon the precipitation of uric acid with an ammoniacal silver solution and magnesia mixture, 1 molecule of silver corresponding to 1 molecule of uric acid. As the amount of silver thus precipitated can be determined by titration with a solution of potassium sulphocyanide, the corresponding amount of uric acid is readily found.

Solutions required: 1. An ammoniacal silver solution. 2. An ammoniacal magnesia mixture. 3. A one-fiftieth normal solution of silver nitrate. 4. A one-fiftieth normal solution of potassium sulphocyanide.

Preparation of these solutions:

1. The ammoniacal silver solution is prepared by dissolving 26 grammes of silver nitrate in distilled water, and adding enough ammonia to redissolve the brown precipitate of argentic oxide which is first formed; distilled water is then added in sufficient amount to make the total quantity 950 c.c. This solution is brought to the proper strength by titrating a known amount of sodium chloride, as described elsewhere. Each cubic centimeter then contains 0.026 gramme of silver nitrate, which is equivalent to 0.0169 gramme of silver.

2. The ammoniacal magnesia mixture is prepared by dissolving 100 grammes of crystallized magnesium chloride in a sufficient amount of water; to this a cold saturated solution of ammonium chloride is added in excess, and sufficient strong ammonia to impart a decided odor. Should the mixture not be perfectly clear, more ammonium chloride solution is added. The solution is then diluted with water to 1 liter.

3. The one-fiftieth normal solution of silver nitrate is prepared by dissolving 3.4 grammes of silver nitrate in 950 c.c. of distilled water, the degree of further dilution being determined as described elsewhere.

4. To prepare the one-fiftieth normal solution of potassium sulphocyanide, about 2 grammes of the salt are dissolved in 950 c.c. of water; the solution is brought to the required strength, so that 1 c.c. shall correspond to 1 c.c of the silver solution.

For filtering the uric acid, a perforated platinum cone is placed in a small funnel and packed with a thin layer of glass-wool, upon which in turn a layer of finely scraped asbestos is placed. The asbestos is previously thoroughly washed with dilute hydrochloric acid and subsequently with distilled water until every trace of chlorine has disappeared. When properly prepared, the asbestos forms a mould of the cone.

Method.—Five c.c. of the ammoniacal silver solution are mixed with 5 c.c. of the ammoniacal magnesia mixture. Ammonia is then added until the solution is clear, when it is poured into 50 c.c. of urine. As soon as the precipitate has settled the supernatant liquid

[1] Haycraft, Zeit. f. analyt. Chem., vol. xxv.

is passed through the prepared filter with the aid of a suction-pump. About 4 grammes of sodium bicarbonate in coarse pieces are now placed upon the filter and the precipitate added; the sodium bicarbonate serves the purpose of aiding filtration by loosening the precipitate. This is now washed free from chlorine and silver by means of ammoniacal water, using the suction-pump until the precipitate appears broken in places, then without the pump, using this only at last to remove the last drops of liquid. (Test for silver with very dilute hydrochloric acid, and for chlorine with a solution of silver nitrate and nitric acid.) The precipitate is now dissolved on the filter by means of nitric acid of 20 to 30 per cent. The nitric acid must be free from nitrous acid. This is secured by allowing it to stand in contact with pure urea until any evolution of gas has ceased. The filter is washed with very dilute nitric acid and then with distilled water until this no longer shows an acid reaction. The solution thus obtained is titrated with the one-fiftieth normal solution of potassium sulphocyanide, using ammonio-ferric alum as an indicator. As each cubic centimeter of this solution indicates 0.0169 gramme of silver, and as 1 molecule of silver indicates 1 molecule of uric acid—*i. e.*, 108 grammes of silver 168 grammes of uric acid—0.0169 gramme of silver, corresponding to 1 c.c. of the potassium sulphocyanide solution, represents 0.2629 gramme of uric acid.

Ludwig-Salkowski Method.—*Principle.*—The method is based upon the formation of insoluble magnesium-silver urate when a solution of uric acid in sodium carbonate is treated with a solution of silver nitrate after the previous addition of an excess of ammonia. This is then decomposed, with the liberation of free uric acid.

Method.[1]—Two hundred and fifty c.c. of urine are treated with 50 c.c. of ammoniacal magnesia mixture (see above) to remove the phosphates. The magnesia mixture is employed for the reason that the compound of uric acid with magnesium and silver which is formed later on is not decomposed so easily as the sodium or the potassium compound, which would occur if the urine were precipitated only with ammonia. The mixture is then immediately filtered, as otherwise a little magnesium urate would be precipitated. Two hundred and forty c.c. of the filtrate, corresponding to 200 c.c. of urine, are measured off as soon as possible, and treated with a few cubic centimeters of a 3 per cent. solution of silver nitrate. If the precipitated silver chloride formed in the beginning does not disappear on stirring, a little more ammonium hydrate is added. A flaky precipitate next separates out, and is allowed to settle. In order to test whether enough of the silver nitrate solution has been added, a few cubic centimeters of the supernatant fluid are acidified with nitric acid. If a distinct cloudiness, referable to silver

[1] E. Salkowski, Salkowski u. Leube, Die Lehr vom Harn. E. Ludwig, Wien. med. Jahrbücher, 1884, p. 597.

chloride, appears, enough has been added. Otherwise the few cubic centimeters that were employed for this test are rendered alkaline again with ammonia, poured back, and treated with more silver solution until the required amount has been reached. The liquid is then rapidly filtered through a folded filter of rather loose paper, a feather or rubber-tipped glass rod being used for the purpose of removing all the precipitate from the beaker. The precipitate is washed with ammoniacal water until a specimen of the washings is no longer rendered turbid by nitric acid, and only faintly so by the addition of a drop of silver solution. The filter with the precipitate is next placed in a wide-mouthed flask, containing about 200 c.c. of distilled water, and thoroughly agitated. Hydrogen sulphide is then passed through the mixture. It is now brought to the boiling-point and rendered distinctly acid by means of a few drops of hydrochloric acid, when the argentic sulphide and the paper are *rapidly* filtered off, as otherwise there will be an admixture of sulphur with the uric acid. The contents of the filter are washed a few times with hot water. Filtrate and washings are quickly evaporated to a few cubic centimeters, treated with a few drops of hydrochloric acid, and set aside in a cool place for twenty-four hours. Occasionally it happens that upon addition of the hydrochloric acid a cloudiness appears, which is due to an admixture of sulphur. In such a case the dried uric acid must be washed with carbon disulphide. Otherwise the uric acid that has separated out is directly collected on a dried and weighed filter, and washed successively with water, 90 to 94 per cent. alcohol, and finally with absolute alcohol and ether. The water used in washing should be collected separately, and for each 20 c.c. used 0.0048 gramme added to the weight of the uric acid obtained.

Precautions: 1. Rapidity in working is essential.

2. Very concentrated urines must be diluted one-half before commencing the examination.

3. If the specific gravity of the urine is low, it should be concentrated to a specific gravity of about 1.020.

4. If the urine shows a sediment of uric acid, this should be separately collected and weighed, and the weight obtained added to the final result.

5. Any albumin that may be present must be previously removed.

6. If sugar is present in the urine, about 500 to 1000 c.c. are treated with a solution of neutral lead acetate, filtered, and the filtrate precipitated with mercuric acetate. The precipitate thus formed, which consists essentially of mercuric urate, is filtered off after having stood for twelve to twenty-four hours, then washed, and later suspended in water. The mercury is removed by means of hydrogen sulphide, the mercuric sulphide filtered off, and the filtrate collected and set aside. The precipitate itself is thoroughly boiled with water and again filtered, the washings thus obtained being

added to the filtrate set aside, as just described. The total amount of fluid is then evaporated to a small volume and acidified with hydrochloric acid, when the uric acid will separate out and may be treated as previously directed.

The Xanthin-bases.

The xanthin-bases which have been found in the urine are xanthin, hypoxanthin, heteroxanthin, paraxanthin, guanin, and adenin. Conjointly they are also spoken of as the alloxur bases, or purin bases. Together with uric acid they are termed alloxur or purin bodies. Their relation to uric acid and the nucleins has already been considered (see page 442). Unlike uric acid, they also occur as such in animal as well as vegetable tissues. The amount which appears in the urine under normal conditions is very small, constituting about 10 per cent. of the uric acid. Larger quantities may be met with in various diseases, and, generally speaking, an increase in the amount of uric acid is associated with an increase of the xanthin-bases. This is, however, not invariably the case, and at times it may be observed that an increase of the uric acid is accompanied by a diminution of the xanthins, and *vice versa*. These varying relations can, of course, be readily understood if we remember that uric acid is an oxidation-product of the xanthin-bases, and that their ultimate origin is the same.

The literature which deals with the elimination of the xanthin-bases in various diseases has during the past few years assumed enormous proportions. This has largely been owing to the publication by Krüger and Wulff of a relatively simple method for their quantitative estimation. Unfortunately, however, this method has proved unreliable and the results obtained incorrect. Our knowledge of the relation of the xanthins to pathological processes is hence as defective at the present time as it was years ago.

Individually the xanthin-bases are of little clinical interest. Xanthin has once been found in a urinary sediment, and has in several instances been encountered as the principal constituent of vesical calculi. Its normal quantity is said to vary between 0.02 and 0.03 gramme. Larger quantities are found after a meal rich in nucleins, in leukæmia, nephritis, pneumonia, etc.

Paraxanthin and heteroxanthin are present only in traces, as is apparent from the fact that Krüger and Salomon were able to obtain but 7.5 grammes of heteroxanthin from 10,000 liters of urine. Both apparently are distinctly toxic.

Xanthin sediments may be recognized by means of the following test: a small amount of the material is treated with a few drops of concentrated nitric acid on a porcelain plate, and evaporated to dryness. In the presence of xanthin a yellow residue is obtained, which turns red upon the addition of a few drops of sodium hydrate solu-

tion and the application of heat. The reaction is common to all the xanthins.

Quantitative Estimation.—Salkowski's Method.[1]—Six hundred c.c. of urine are precipitated with 200 c.c. of magnesia mixture (see page 401), when a 3 per cent. ammoniacal solution of silver nitrate is added to from 700 to 750 c.c. of the filtrate. The proportion should be 6 c.c. for each 100 c.c. of urine. The silver nitrate solution should be added as described on page 453. After standing for one hour the mixture is filtered, and the precipitate washed with water until all the free silver has been removed. The filter is then perforated, the precipitate washed into a flask with from 600 to 800 c.c. of water, acidified with hydrochloric acid, and decomposed with hydrogen sulphide. The excess of hydrogen sulphide is removed by heating on a water-bath, when the silver suphide is filtered off and the filtrate evaporated to dryness. The residue is treated with from 25 to 30 c.c. of dilute sulphuric acid (1 : 100). This solution is brought to the boiling-point and is allowed to stand over night. The uric acid which has then separated out is filtered off, washed with a small amount of dilute sulphuric acid (not more than 50 c.c.), then with alcohol and ether, and weighed. To the resulting weight 0.0005 gramme is added for each 10 c.c. of the acid filtrate, to allow for the trace of uric acid which is thus lost.

After having filtered off the uric acid the filtrate is again treated with ammonia and silver solution, and the xanthin-bases thus precipitated. The precipitate is collected on a small filter, washed with water, dried, and incinerated. The ash is dissolved in nitric acid, and the silver estimated by titration with a solution of potassium sulphocyanide, using ammonio-ferric alum as an indicator (see page 393). The solution of potassium sulphocyanide employed in the estimation of the chlorides may be used, and is of such strength that 1 c.c. corresponds to 0.00734 gramme of silver. As 1 atom of silver in a mixture of the silver compounds of guanin, xanthin, hypoxanthin, etc., represents 0.277 gramme of nitrogen, or 0.7381 gramme of the alloxur bases, it is apparent that 1 c.c. of the potassium sulphocyanide solution will represent 0.002 gramme of nitrogen and 0.00542 gramme of alloxur bases. In every case an accurate record must, of course, be kept of the amount of urine and filtrate used.

The amount of alloxur bases found by Salkowski in the normal urine of twenty-four hours varied between 0.0286 and 0.0561 gramme.

LITERATURE.—M. Krüger u. G. Salomon, "Die Alloxurbasen d. Harns," Zeit. f. physiol. Chem., vol. xxiv. p. 364, and vol. xxvi. p. 343; Deutsch. med. Woch., 1899, p. 97. Bondsynski u. Gottlieb, "Ueber Xanthinkörper im Harn des Leukämiker," Arch. f. exper. Path. u. Pharmakol., 1895, vol. xxxvi. p. 132. F. Gumprecht, "Alloxurkörper u. Leukocyten," Centralbl. f. allg. Path. u. path. Anat., 1896, vol. vii. p. 820.

[1] E. Salkowski, Pflüger's Archiv, vol. lxix. p. 268.

Hippuric Acid.

Hippuric acid is a constant constituent of normal urine, 0.1 to 1 gramme being excreted in the twenty-four hours. That it is derived, to some extent at least, from albuminous material is proved by the fact that its elimination is not suspended during starvation nor during the administration of a purely albuminous diet. The manner in which hippuric acid is formed in the body-economy, however, has not been definitely ascertained. *In vitro* it may be obtained from glycocoll and benzoic acid, according to the equation

$$\underset{\text{Benzoic acid.}}{C_6H_5COOH} + \underset{\text{Glycocoll.}}{CH_2NH_2COOH} = \underset{\text{Hippuric acid.}}{CH_2NH-C_6H_5COCOOH} + H_2O.$$

It has been shown that phenyl-propionic acid, which differs from benzoic acid by the group C_2H_4, and which latter may be regarded as phenyl-formic acid, is produced during the process of intestinal putrefaction. The relation between the two bodies is seen from the formulæ:

$$\underset{\text{Formic acid.}}{H-COOH} \longrightarrow \underset{\text{Phenyl-formic acid.}}{C_6H_5-COOH}$$

$$\underset{\text{Propionic acid.}}{CH_3-CH_2-COOH} \longrightarrow \underset{\text{Phenyl-propionic acid.}}{CH_2.C_6H_5-CH_2-COOH}$$

Phenyl-propionic acid is then absorbed into the blood and there, according to our present ideas, transformed into phenyl-formic acid or benzoic acid. When the latter comes in contact with glycocoll, which is probably also produced during the process of intestinal putrefaction, an interaction between the two substances occurs in the body, hippuric acid resulting, as shown in the above equation. This view is supported by the fact that phenyl-propionic acid, just as benzoic acid, when introduced into the circulation of certain animals, reappears in the urine as hippuric acid. The final proof of the possible synthesis of hippuric acid from glycocoll and benzoic acid in the body has been furnished by Bunge and Schmiedeberg,[1] who obtained this substance, when arterialized blood containing glycocoll and sodium benzoate was allowed to pass through isolated kidneys of dogs.

Not all the hippuric acid eliminated, however, is referable to albuminous decomposition, but a considerable portion is derived from benzoic acid or its derivatives, which occur in many fruits, and are transformed into hippuric acid in the body. Among those which are particularly rich in these substances may be mentioned

[1] Schmiedeberg u. Bunge, Arch. f. exper. Path. u. Pharmakol., vol. vi.

the red bilberry, prunes, coffee-beans, reinesclaudes, etc., and in all cases in which an increased elimination of hippuric acid is observed the possibility of this source must always be taken into account.

As to the seat of this synthesis there appears to be some uncertainty, as it is apparently not the same in all animals. In the dog and the frog the kidneys, according to the researches of Bunge and Schmiedeberg, must be regarded as the principal and possibly the only organs in which this process occurs. As Salomon, however, has demonstrated the presence of hippuric acid in the muscles, liver, and blood of nephrectomized rabbits, still other organs must, in the herbivora at least, be concerned in its production.

Very little is known of the pathological variations in the excretion of hippuric acid; this is principally owing to the fact that until recently suitable methods for its quantitative estimation were not available. It is an interesting fact that, in accordance with Bunge's experiments in dogs, the formation of hippuric acid appears to be suspended in cases of acute as well as chronic parenchymatous nephritis, for the benzoic acid which is then ingested reappears in the urine unchanged. In amyloid degeneration a marked diminution in its amount has likewise been demonstrated. Large quantities of hippuric acid, on the other hand, have been noted in acute febrile diseases, hepatic diseases, diabetes mellitus, chorea, etc. The data, however, are insufficient to warrant any definite conclusions at the present time.[1]

Properties of Hippuric Acid.—Chemically, hippuric acid must be regarded as benzoyl-amido-acetic acid, $C_9H_9NO_3$—(C_6H_5.CONH.CH_2.COOH). It crystallizes in long rhombic prisms when allowed to separate from its solutions gradually, while it forms long needles if crystallization takes place rapidly and the amount is small (Fig. 95). In cold water and ether it is soluble with difficulty, while it dissolves readily in hot water, in alcohol, and in aqueous solutions of the hydrates and carbonates of the alkalies, with which it forms salts, and from which the acid may again be separated and caused to crystallize out by adding a stronger acid.

When hippuric acid or one of its salts is evaporated to dryness with concentrated nitric acid and the residue is heated, the odor of bitter almonds is noticed; this is due to the formation of nitrobenzol.

When boiled with hydrochloric acid or dilute sulphuric acid hippuric acid is decomposed into glycocoll and benzoic acid. A similar decomposition is effected during the process of putrefaction, and hence no hippuric acid is found in decomposing urine, *benzoic acid* taking its place. The latter is always found in the urine together with hippuric acid, but has no clinical significance. In

[1] Th. Weyl u. B. von Anerep, "Ueber die Ausscheidung der Hippursäure und Benzoesäure während des Fiebers," Zeit. f. physiol. Chem., 1880, vol. iv. p. 169.

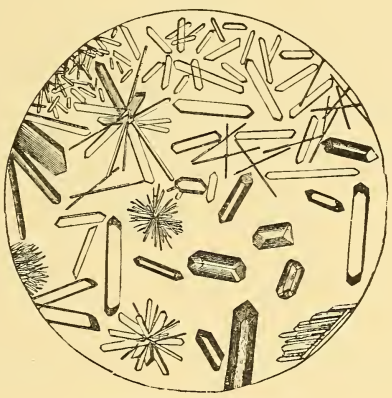
larger amounts it has recently been encountered in a case of diabetes. It crystallizes in needles or lustrous laminæ, presenting ragged edges, which resemble plates of cholesterin. It is soluble with difficulty in cold water, but easily soluble in ether, alcohol, and solutions of the alkaline carbonates and hydrates, forming salts with the latter.

Hippuric acid in the urine occurs in combination with sodium, potassium, calcium, and magnesium.

Quantitative Estimation of Hippuric Acid.—The following method, which may be employed for the quantitative estimation of hippuric acid, although tedious, must also be employed when it is desired to test for its presence.

Principle.—Hippuric acid readily dissolves in solutions of the alkaline hydrates and carbonates, forming salts. These are decomposed by means of a stronger acid, when the hippuric acid which separates out is collected and weighed.

Fig. 108.



Hippuric acid crystals.

Method.—Five hundred to 1000 c.c. of fresh urine are evaporated to a syrupy consistence on a water-bath, care being taken to keep the urine neutral by the addition of sodium carbonate. The residue is extracted with cold alcohol (90 to 95 per cent.), using about half of the quantity as that of the urine employed. The mixture is then set aside for twenty-four hours. The alcoholic filtrate, which contains the salts of hippuric acid, is freed from alcohol by distillation. The remaining solution is strongly acidified with acetic acid and extracted with at least five times its volume of alcoholic ether (1 part of alcohol to 9 parts of ether). From the combined extracts the ether is distilled off and the remaining solution evaporated on a water-bath. The resinous residue is boiled with water, set aside to cool, and filtered through a well-moistened

filter. The hippuric acid, which is easily soluble in boiling water, is thus separated from other constituents which are soluble in alcohol and ether. The filtrate is rendered alkaline with a little milk of lime, any excess of calcium being removed by passing carbon dioxide through the mixture. This is then brought to the boiling-point and filtered. Any impurities which may be present are removed by shaking with ether. The calcium salts remaining in solution are decomposed by means of an acid, when the solution is again extracted with ether. The remaining solution is evaporated to a few cubic centimeters, when the hippuric acid will separate out on standing. The crystals are dried on plates of plaster of Paris, shaken with benzol or petroleum-ether to remove any benzoic acid, and finally weighed. These crystals may be shown to be hippuric acid by their microscopical appearance, their solubility in alcohol, and their behavior when evaporated with concentrated nitric acid, as indicated above.

Hofmeister's Method.—Two hundred to 300 c.c. of urine are evaporated in a glass dish to one-third of the original volume, and treated with 4 grammes of disodium phosphate, to transform the acid into its sodium salt. The mixture is evaporated to a syrupy consistence, the residue treated with burnt gypsum, dried thoroughly, and pulverized together with the dish. The powder is extracted in a Soxhlet apparatus with freshly rectified petroleum-ether (boiling-point 60° to 80° C.) for forty-six hours, and then for six to ten hours with pure ether (free from water and alcohol). After distilling off the ether the residue is dissolved in boiling water and decolorized with animal charcoal, the latter being subsequently thoroughly washed with boiling water; the solution and washings are evaporated to about 1 or 2 c.c. at a temperature of from 50° to 60° C., and set aside to crystallize. The crystals of hippuric acid are finally washed with a few drops of water and ether, and weighed.

Kreatin and Kreatinin.

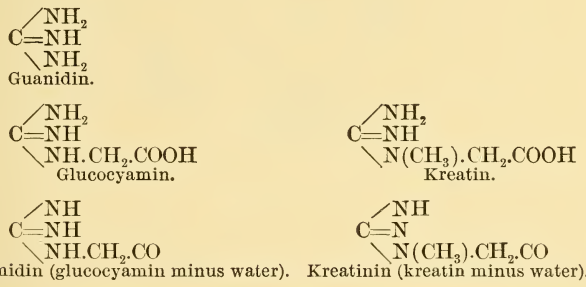
Kreatin, which is constantly present in muscle-tissue, is in all probability the immediate and constant antecedent of kreatinin, so that two sources of this body must be recognized, viz., the muscle-tissue of the body and the muscle-tissue ingested as food. Beyond this, however, practically nothing is known, and as the artificial production of kreatinin from albuminous material has never been accomplished, it is hardly warrantable to venture an hypothesis as to its mode of formation in the body.

Kreatinin is a constant constituent of the urine, about 1 gramme being excreted daily by a healthy adult. Pathologically, variations in this amount have been observed, but the data obtained possess little value. Before drawing conclusions from observations made in

the clinical laboratory it is necessary to take into account the quantity of meat ingested, as a meat-diet will greatly increase the amount of kreatinin. If then in patients affected with acute febrile diseases, such as pneumonia, typhoid fever, etc., a large increase is observed, the patient being at the same time upon a milk-diet, an increased destruction of muscle-tissue may be inferred, as a milk-diet in itself, *cæteris paribus*, causes a diminished elimination. A decrease would logically be expected to occur during convalescence from such diseases. In the various forms of anæmia, marasmus, chlorosis, phthisis, etc., a diminished amount is observed.[1]

The transformation of kreatin into kreatinin has been supposed to take place in the kidneys, a view which accords with the greatly diminished excretion of kreatinin in advanced cases of chronic parenchymatous nephritis. In progressive muscular atrophy, in pseudohypertrophic paralysis, and in progressive ossifying myositis a diminution has also been noted.

Properties of Kreatin and Kreatinin.—Chemically, kreatin may be regarded as a methyl derivative of glucocyamin, which latter is guanidin in which 1 NH_2 group has been replaced by glycocoll. Kreatinin, on the other hand, is the methyl derivative of glucocyamidin, which differs from glucocyamin only in the absence of 1 molecule of water, so that kreatinin is kreatin minus 1 molecule of water, both being derivatives of guanidin. The relation between the various bodies is shown below:



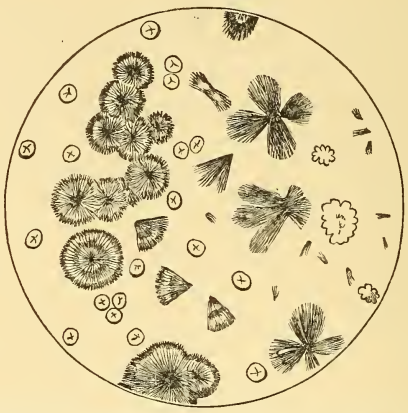
Guanidin. Glucocyamin. Kreatin. Glucocyamidin (glucocyamin minus water). Kreatinin (kreatin minus water).

Kreatinin crystallizes without water of crystallization in colorless, glistening prisms. At times, when the crystals are not well developed, it also appears in the form of whetstones. It is readily soluble in hot and also quite soluble in cold water and hot alcohol; in cold alcohol and ether it dissolves with difficulty. It forms salts with acids, and double salts with some of the salts of the heavy metals. Among these may be mentioned kreatinin hydrochloride, $C_4H_7N_3O.HCl$, which is easily soluble in water and crystallizes in the form of transparent prisms or rhombic plates. Most important is the com-

[1] C. E. Simon, Physiological Chemistry, Lea Bros. & Co., 1901. Senator, Virchow's Archiv, 1876, vol. lxvii. p. 422. Neubauer u. Vogel, Harnanalyse, Pt. ii.

pound of kreatinin with zinc chloride, $(C_4H_7N_3O)_2.ZnCl_2$ (Fig. 109). This is produced when a watery or alcoholic solution of kreatinin is treated with zinc chloride. The crystalline form of this compound depends greatly upon the purity of the kreatinin solution. When obtained from alcoholic extracts of the urine it occurs in the form of varicose conglomerations which often adhere firmly to the walls of the vessel. If the solution of kreatinin is perfectly pure, however, it is seen in the form of fine needles grouped in rosettes or sheaves. Kreatinin-zinc chloride is soluble with much difficulty in water and insoluble in alcohol. The compound is especially important, as upon its formation and properties the quantitative estimation of kreatinin in the urine is based. Silver nitrate and mercuric chloride cause a precipitation of kreatinin, and may, therefore, also be employed for the purpose of obtaining the substance from the urine.

FIG. 109.



Crystals of kreatinin-zinc chloride. (SALKOWSKI.)

Test for Kreatinin in the Urine.—A few cubic centimeters of urine are treated with a few drops of a very dilute solution of sodium nitroprusside and then drop by drop with a dilute solution of sodium hydrate. In the presence of kreatinin the urine assumes a ruby-red color, which is particularly well seen in the lower portion of the tube. This color disappears after a few minutes, and is replaced by an intense yellow, which on warming with glacial acetic acid in pure solutions turns to green, then to blue, and on standing a deposit of Prussian blue is obtained (*Weyl's test*).[1] The presence of albumin or sugar does not interfere with the reaction.

Quantitative Estimation of Kreatinin in the Urine.[2]—*Principle.*—When an alcoholic extract of urine is treated with an alco-

[1] Th. Weyl, Ber. d. deutsch. chem. Gesellsch., 1878, vol. xi. p. 217; and Jaffé, Zeit. f. physiol. Chem., 1886, vol. x. p. 399.

[2] Leube u. Salkowski, Die Lehre vom Harn, Hirschwald, Berlin, 1882, p. 111.

holic solution of zinc chloride kreatinin-zinc chloride separates out. This, as has been mentioned, is almost insoluble in alcohol. Knowing the molecular weight of kreatinin and kreatinin-zinc chloride, the calculation of the amount of kreatinin becomes a simple matter. The molecular weight of kreatinin is 113, that of kreatinin-zinc chloride 362. In 362 parts by weight of the latter there are, hence, 226 parts by weight of kreatinin, so that the amount of the kreatinin may be calculated from the weight of the kreatinin-zinc chloride according to the following equation: $362 : 226 :: y : x$; and $x = 0.6243\,y$, in which y indicates the weight of the kreatinin-zinc chloride found, and x the corresponding amount of kreatinin. The phosphates must, of course, first be eliminated, as insoluble zinc phosphate would otherwise be precipitated.

METHOD.—In 200 c.c. of urine the phosphates are first removed by alkalinizing with milk of lime, and then adding calcium chloride so long as a precipitate forms. If the volume is now less than 300 c.c., water is added to that amount. The mixture is filtered after having been allowed to stand for from one-quarter to one-half hour, and washed with a little water. Filtrate and washings are slightly acidified with dilute hydrochloric acid, so as to prevent the transformation of kreatinin into kreatin, and evaporated on a water-bath to a syrupy consistence, and then thoroughly mixed with 20 to 30 c.c. of absolute alcohol. The mixture is poured into a stoppered flask provided with a 100 c.c. mark, and after thoroughly rinsing out the evaporating-dish with absolute alcohol the washings are also placed in the bottle, and absolute alcohol is added to the mark. The bottle is thoroughly shaken and set aside in a cool place for twenty-four hours, the mixture being agitated from time to time. It is now filtered and rendered slightly alkaline with a drop or two of a sodium carbonate solution, as kreatinin hydrochloride is not precipitated by zinc chloride. The reaction, however, should be only *faintly* alkaline, as otherwise zinc oxide will be precipitated. The mixture is now slightly acidified with acetic acid and treated with 0.5 c.c. of an alcoholic solution of zinc chloride, prepared by dissolving the salt in 80 per cent. alcohol and diluting with 95 per cent. alcohol to a specific gravity of 1.2. The mixture is well stirred and set aside in a cool place for two or three days. The crystals, which are usually deposited on the sides of the vessel in the form of wart-like masses, are then collected upon a dried and weighed filter, always using portions of the filtrate to bring the crystals completely upon the filter. These are washed with a small amount of 90 per cent. alcohol, until the washings are without color and give only a slight opalescence when treated with a drop of silver nitrate solution. The crystals are finally dried at a temperature of 100° C., and weighed. By multiplying the weight thus found by 0.6243 the amount of kreatinin is obtained.

Precautions: 1. Albumin and sugar, if present, must first be removed. In diabetic urines it is best, after having removed the sugar by fermentation, to take one-fifth of the total quantity eliminated in twenty-four hours, and to evaporate this to about 300 c.c. before removing the phosphates.

2. The weighed material should be examined microscopically, to see whether notable quantities of sodium chloride are present. Should this be the case, it is necessary to determine the amount of zinc present, and to estimate the kreatinin from this. To this end, the kreatinin-zinc chloride is evaporated to dryness after the addition of a little nitric acid. The residue is incinerated, extracted with water, washed, dried, fused, and finally weighed.

As 100 parts of kreatinin-zinc chloride correspond to 22.4 parts by weight of zinc oxide, the corresponding amount of the compound is found according to the following equation: $22.4 : 100 :: y : x$; and $x = 4.4642\ y$, in which y represents the amount of zinc oxide found, and x the corresponding amount of kreatinin-zinc chloride. By multiplying the number thus ascertained by 0.6243 the amount of kreatinin is found.

3. Instead of doing this, the precipitate in the alcoholic solution may be examined microscopically before filtering. If sodium chloride crystals are found, providing that the kreatinin-zinc chloride adheres to the sides of the vessel, the sodium chloride may be dissolved in a little water and poured off.

4. If the crystals of kreatinin-zinc chloride adhere very firmly to the sides of the vessel, so that their removal would be incomplete, it is perhaps best to dissolve them in a little hot water, to evaporate to dryness, and to weigh the kreatinin compound directly.

5. If the urine shows an alkaline reaction, it is best to acidify with sulphuric acid, and to boil for half an hour before removing the phosphates, so as to transform any kreatin that may be present into kreatinin, when the examination is continued as described.

Folin's Method.[1]—This method is based on Jaffé's reaction of kreatinin with alkaline picric acid solution. The red colored solution produced in this reaction has in proper concentration and when viewed by transmitted light exactly the same shade as a potassium bichromate solution. Half-normal potassium bichromate solution (containing 24.55 grammes per liter) is therefore used as a standard for comparison.

A high-grade colorimeter, by means of which the depths both of the unknown solution and of the bichromate can be adjusted to tenths of millimeters, is necessary for the comparison.[2]

[1] The above description of the as yet unpublished method I owe to the courtesy of O. Folin.

[2] The French instrument of *Duboscq*, which can be obtained through *Eimer & Amend*, is admirably suited for the purpose.

The following solutions are also necessary: The half-normal potassium bichromate solution, 10 per cent. sodic hydrate, and a saturated (1.2 per cent.) picric acid solution.

If to 10 mgrms. of chemically pure kreatinin dissolved in 10 c.c. of water in a 500 c.c. volumetric flask are added 15 c.c. of picric acid solution and 5 c.c. of sodic hydrate, the maximum color is obtained at the end of five minutes. If at the end of this time the solution be diluted to the 500 c.c. mark and at once compared with the standard bichromate solution, it will be found that 8.1 mm. of the kreatinin-picrate solution have in the colorimeter exactly the same shade and depth of color as 8 mm. of the bichromate solution.

The actual determination in urine is carried out in exactly the same way, substituting 10 c.c. of urine for the kreatinin solution. The more kreatinin that is present in the 10 c.c. of urine the deeper will, of course, be the color of the solution obtained. Supposing the colorimetric observation shows that 7.1 mm. of the urine picrate solution are equal in color to 8 mm. of the standard. The 10 c.c. of urine would then contain $10 \times \frac{8.1}{7.1} = 11.4$ mgrms. of kreatinin.

The following precautions are to be observed in the determination:

1. Make first a preliminary colorimetric observation, using half-normal potassium bichromate solution in both cylinders of the colorimeter, adjusting first one to the 8 mm. mark. The average of three or four readings of the other cylinder should also be 8 mm., and after the first observation no two should differ by more than 0.2 mm. This preliminary observation takes only two or three minutes, and is exceedingly useful in making the eye sure of the correct point to be ascertained.

2. Exactly 8 mm. of the half-normal potassium bichromate solution must be used as the standard for comparison. 16 or 24 mm., for example, cannot be substituted on the basis of the calculation given above because the kreatinin picrate solution absorbs light at an entirely different rate from that of the bichromate solution.

3. For the reason given in the preceding paragraph it is necessary to make each determination with a quantity of urine containing not less than 5 nor more than 15 mgrms. of kreatinin. Within these limits the determination as described is correct within 0.2 mgrm.

4. Sugar and albumin do not interfere with the determination. Acetone, diacetic acid, and hydrogen sulphide do interfere. Where these are present the urine should be measured into a porcelain evaporating-dish and heated on a water-bath with 10 c.c. of 1 per cent. hydrochloric acid for about half an hour. When the dish is again cooled, the reagents are added directly into the dish, and finally rinsed into the volumetric flask after five minutes.

5. The color due to the urine is ordinarily of no appreciable con-

sequence because of the great dilution. Urines containing bile-pigments can, however, first be cleared by the addition of egg-albumin and then removing this by coagulation (heat).

The whole operation can be finished in less than fifteen minutes; indeed, it should be finished at once, as the colored product obtained by the interaction of kreatinin and picric acid is not very stable.

Oxalic Acid.

The origin of oxalic acid in normal urine is twofold. The greater portion is supposedly derived from the ingested food, but there is evidence to show that a certain amount is also formed during the metabolism of the body-tissues, as the elimination of oxalic acid does not cease during starvation. The carbohydrates and fats probably do not play a part in this connection; and, according to Salkowski, the albumins also do not enter into consideration *per se*. He rather inclines to the view that the nucleins represent the antecedent of the oxalic acid, and as a matter of fact uric acid, which, as we have seen, is itself derived from the nucleinic bases, can be readily oxidized to oxalic acid, with the intermediary formation of parabamic acid and *oxaluric acid*. The latter has been repeatedly demonstrated in the urine, and it is conceivable that the same process may occur in the animal body. But even supposing that the oxaluric acid which is obtained from the urine is formed artificially during the lengthy process of analysis, and that the substance did not exist preformed, there is no reason for the assumption that uric acid may not be the normal antecedent of the oxalic acid. For Salkowski has demonstrated conclusively that on oxidation with ferric chloride in aqueous solution uric acid yields oxalic acid and urea directly. These various changes may be expressed by the equations:

(1) $C_5H_4N_4O_3 + H_2O + 2O = CO\langle \begin{matrix} NH.CO \\ | \\ NH.CO \end{matrix} \rangle + CO\langle \begin{matrix} NH_2 \\ NH_2 \end{matrix} + CO_2$

Uric acid. Parabanic acid. Urea.

(2) $CO\langle \begin{matrix} NH.CO \\ | \\ NH.CO \end{matrix} \rangle + H_2O = \begin{matrix} CO.NH.CONH_2 \\ | \\ COOH \end{matrix}$

Parabanic acid. Oxaluric acid.

(3) $\begin{matrix} CO.NH.CONH_2 \\ | \\ COOH \end{matrix} + H_2O = \begin{matrix} CO.OH \\ | \\ CO.OH \end{matrix} + CO\langle \begin{matrix} NH_2 \\ NH_2 \end{matrix}$

Oxaluric acid. Oxalic acid. Urea.

(4) $C_5H_4N_4O_3 + 3H_2O + 2O = \begin{matrix} CO.OH \\ | \\ CO.OH \end{matrix} + 2CO\langle \begin{matrix} NH_2 \\ NH_2 \end{matrix} + CO_2$

Uric acid. Oxalic acid. Urea.

The matter, however, is not quite so simple as it appears, and an increased elimination of oxalic acid by no means always occurs when the output of uric acid is increased. After the ingestion of fairly large amounts of thymus, for example, the usual increase of uric acid is not accompanied by a corresponding increase in the amount of oxalic acid, and in those cases in which it does occur we are as yet unable to exclude the large amount of connective tissue as the source of the oxalic acid. Connective tissue and gelatin have, as a matter of fact, been shown to increase the amount of oxalic acid when given in large amounts. With pure nuclein no effect has been observed, and it can be shown that in those experiments in which this was used by mouth an absorption from the intestinal tract had manifestly not occurred (Mohr and Salomon).[1]

Under pathological conditions oxalic acid may also be formed in the digestive tract from the ingested carbohydrates, as a result of a peculiar fermentative process. This has been well shown by Helen Baldwin in Herter's laboratory. In some of these cases no free hydrochloric acid could be demonstrated in the gastric contents, and it was observed that inoculation of a digestive mixture, which was originally free from oxalic acid, resulted in its appearance if a few drops of such stomach contents were added. In dogs prolonged feeding with excessive quantities of glucose together with meat was seen to lead eventually to a state of oxaluria, which was associated with a mucous gastritis and the absence of free hydrochloric acid. Oxalic acid could then also be demonstrated in the stomach contents.

Very curiously the ingestion of quite small and non-toxic amounts of oxalic acid is followed by a fairly intense indicanuria. It does not seem likely to me, however, that as Harnack and v. d. Leyen suggest, the indicanuria is here referable to a toxic action upon the tissue-albumins, and I am personally inclined to explain the phenomenon upon the basis of increased intestinal putrefaction (see Indicanuria).

The amount of oxalic acid which is normally eliminated in the twenty-four hours fluctuates with the amount ingested, and varies from a few milligrammes to 2 or 3 centigrammes, being usually less than 10 milligrammes (Baldwin). It is influenced by the character of the diet. The ingestion of oxalates by the mouth is followed only by their partial elimination in urine and feces, so that we may conclude that to a certain extent oxalic acid is decomposed during its passage through the animal body; possibly this may occur in the intestinal canal as the result of bacterial action.

Foods rich in oxalic acid are spinach, tomatoes, carrots, celery,

[1] L. Mohr and H. Salomon, Deutsch. Arch. f. klin. Med., 1901, vol. lxx. p. 486. Lommel, Ibid., vol. lxiii. p. 599.

string-beans, rhubarb, potato, dried figs, plums, strawberries, cocoa, tea, coffee, and pepper. Foods which contain little or no oxalic acid, on the other hand, are meat, milk, eggs, butter, corn-meal, rice, peas, asparagus, cucumbers, mushrooms, onions, lettuce, cauliflower, pears, peaches, grapes, melons, and wheat, rye, and oat flour.

Before drawing conclusions as to the existence of abnormal oxaluria it is hence imperative to eliminate the possibility of an increased ingestion, by placing the patient upon a diet which contains little or no oxalic acid.

An increased elimination is notably observed in association with various dyspeptic and nervous manifestations, and constitutes the condition commonly spoken of as the *oxalic acid diathesis*, or as *idiopathic oxaluria*. Its existence as a definite pathological picture is, however, denied by most modern clinicians. Nevertheless it must be admitted that there is a certain type of neurasthenia in which, generally in association with hyperchlorhydria, an increased elimination of oxalic acid takes place, and in which a copious deposit of calcium oxalate crystals is frequently observed. From the mere fact of the occurrence of such deposits, of course, no inference is as rule to be drawn regarding the actual elimination, but its frequent occurrence is in itself of importance, as in such cases a similar separation from the urine may already occur within the urinary passages, and not uncommonly in the pelvis of the kidneys. Not infrequently oxaluria of this type is associated with an increased elimination of uric acid and a mild grade of albuminuria, as has been shown by Senator, v. Noorden, DaCosta, myself, and others. Whether or not the oxaluria in these cases can be explained upon the basis of abnormal fermentations in the gastro-intestinal tract, as is suggested by the observations of Baldwin, remains to be seen. In some this may be the case, but in others I am inclined to associate the oxaluria with the coexistent lithuria, and rather imagine that both conditions may be referable to impairment of the normal oxidation-processes in the liver.

That this explanation holds good also of the apparent vicarious oxaluria which is at times observed in diabetes, appears quite likely.

Fürbringer has reported a case of diabetes in which the elimination of oxalic acid was described as "enormous," and in which oxalic acid could also be demonstrated in the sputum (oxaloptysis). Rausch has recorded a case of mild diabetes, associated with hepatic cirrhosis, in which 1.2 grammes were excreted in twenty-four hours. In most cases of diabetes, on the other hand, an increased oxaluria cannot be demonstrated.

In cases of obesity Kisch found no abnormal degree of oxaluria.

In association with jaundice increased oxaluria has been repeatedly observed, and is probably referable to biliary stasis and

consequent cholæmia, as Salkowski has demonstrated that the bile contains oxalic acid. In pneumonia and leukæmia, in both of which we find as a rule a greatly increased elimination of uric acid, the oxalic acid is not always increased, and sometimes indeed quite low in comparison to the amount of uric acid.

Properties of Oxalic Acid.—Oxalic acid occurs in the urine as calcium oxalate, CaC_2O_4, and is held in solution by the diacid sodium phosphate. It can, hence, be precipitated by diminishing the acidity of the urine by the addition of a little ammonia or by allowing it to stand exposed to the air. When allowed to crystallize out slowly, calcium oxalate occurs in the form of well-defined, strongly refractive octahedra, in which the principal axis of the crystals is placed at right angles to the plane of the microscope slide (Fig. 110). These are very characteristic. Other forms, however, are also quite commonly observed, such as single and double dumb-bells, spheroids and prisms, etc. (Fig. 105). They are insoluble in ammonia and alcohol, almost insoluble in hot and cold water, and very slightly soluble in acetic acid, but dissolve with ease in the mineral acids.

FIG. 110.

Calcium oxalate crystals.

When strongly heated, the salt is decomposed into calcium oxide, carbon dioxide, and carbon monoxide, according to the equation

$$CaC_2O_4 = CaO + CO_2 + CO.$$

Tests for Oxalic Acid.—For the detection of calcium oxalate it is frequently only necessary to examine the sediment of the urine after standing for twenty-four to forty-eight hours. No oxalate crystals, however, may be found even when an abnormally large amount can be demonstrated by chemical methods. In such cases it is usually possible to bring about the crystallization of the salt by carefully neutralizing the urine with a little ammonia. Should this procedure not lead to the desired end, it is best to treat the urine with one-third its volume of 95 per cent. alcohol. The mixture is set aside for twenty-four to forty-eight hours, when the sediment is

centrifugalized and examined with the microscope. This method, Baldwin states, represents a more delicate test for oxalic acid than the complicated methods of quantitative analysis which are available.

Quantitative Estimation.—Heretofore the old method of Neubauer has been in general use, but it is at best unsatisfactory. It is still described at this place, as the more recent methods of Dunlop and Salkowski are as yet but little known. At the same time it must be admitted that these more modern procedures are likewise not free from objections, but they are nevertheless to be preferred to that of Neubauer.

Neubauer's Method.—*Principle.*—The calcium oxalate in the urine is held in solution by the diacid sodium phosphate. If this is removed by means of calcium chloride and ammonia, the calcium oxalate is precipitated. By heating this strongly it is transformed into calcium oxide.

As 56 parts by weight of calcium oxide correspond to 128 parts by weight of calcium oxalate, the amount of the latter can be readily calculated according to the equation : $56 : 128 :: y : x$; and $x = 2.2857\ y$, in which y indicates the amount of calcium oxide found in a given amount of urine, and x the corresponding amount of calcium oxalate. As 1 molecule of oxalic acid, moreover, corresponds to 1 molecule of calcium oxalate, the amount of the former can be found from that of the latter according to the equation : $128 : 90 :: y : x$; and $x = 0.703\ y$, in which y represents the amount of calcium oxalate found, and x the amount of the corresponding acid.

Method.—A large amount of urine (600 to 1000 c.c.) is thymolized, so as to guard against putrefactive processes, and is treated with an excess of calcium chloride solution and rendered strongly alkaline with ammonia. The diacid sodium phosphate which holds the oxalic acid in solution is thus removed. The precipitate of phosphates is then carefully treated with an amount of acetic acid just sufficient to dissolve it, without filtering. As calcium oxalate is almost insoluble in acetic acid, it gradually separates out. To this end, the mixture is allowed to stand for twenty-four hours, the addition of the thymol preventing the development of bacteria. At the end of this time the calcium oxalate is filtered off through a small filter. It is washed with water and treated with a small amount of warm hydrochloric acid, any uric acid that may have separated out being left behind. The filtrate is further treated with a small amount of very dilute ammonia, so as to render the solution *slightly* alkaline. After standing for twenty-four hours the calcium oxalate will have separated out, and is collected upon a smaller filter, the weight of the ash in this being known. After washing with water the contents of the filter are dried and incinerated in a crucible, heating strongly for about twenty minutes, whereby the oxalate

is transformed into the oxide. From the weight of this the corresponding amount of oxalic acid is readily calculated according to directions given above.

Dunlop's Method (slightly modified by Baldwin).—In this case the calcium oxalate is precipitated from an acid solution by means of alcohol, instead of from an alkaline solution by calcium chloride. The urine is thymolized, and if alkaline acidified with a trace of acetic acid.

Five hundred c.c. of a well-mixed specimen of the collected urine of twenty-four hours are treated with 150 c.c. of over 90 per cent. alcohol, to precipitate the calcium oxalate. The mixture is set aside for forty-eight hours. It is then filtered, care being taken to insure the entire removal of the crystals from the beaker. The sediment is thoroughly washed with hot and cold water, and finally with dilute acetic acid (1 per cent. solution). The filter is placed in a small beaker and soaked in a small amount of dilute hydrochloric acid. It is then washed with hot water until the washings no longer give an acid reaction. The acid solution and washings are filtered, and the filtrate evaporated to about 20 c.c. This is treated with a very small amount of a solution of calcium chloride, to insure the presence of an excess of calcium. The solution is neutralized with ammonia, slightly acidified with acetic acid, and treated with strong alcohol, so that the mixture contains 50 per cent. After forty-eight hours the sediment is collected on a filter free from mineral ash, and is washed with cold water and dilute acetic acid until free from chlorides. The filter with its contents is then incinerated, first over a Bunsen burner, and afterward for five minutes in a blow-pipe flame. On cooling over sulphuric acid the ash is weighed; the result multiplied by 1.6 represents the amount of oxalic acid in the volume of urine examined.

Salkowski's Method.—In the case of human urine of moderate concentration 500 c.c. of the non-filtered urine are evaporated to about one-third. On cooling, the liquid is acidified with 20 c.c. of hydrochloric acid (sp. gr. 1.12), and extracted three times with new portions of 200 c.c. each of a mixture of 9 to 10 volumes of ether and 1 volume of alcohol. The ethereal extracts, which contain the liberated oxalic acid, are carefully separated from the urine and filtered through a dry filter. The ether is distilled off; the remaining alcoholic solution, which still contains a little ether, is placed in a deep evaporating-dish, diluted with 10 to 15 c.c. of water, and evaporated on a water-bath. The resulting milky fluid is concentrated, more water being added if necessary, until it becomes clear and a gummy material separates out. On cooling, the liquid, which should measure about 20 c.c., is passed through a small filter. This is washed once or twice with a *little* water, when filtrate and washings are rendered slightly alkaline with ammonia, treated with 1 to 2 c.c.

of a 10 per cent. solution of calcium chloride, and acidified with dilute acetic acid. The reaction should be distinctly acid, but an excess should be avoided. An indication that a sufficient amount has been added is afforded by the dissolution of the precipitate of phosphates, which occurs after the addition of the calcium chloride solution. After standing for twenty-four hours, or still better forty-eight hours, the calcium oxalate that has separated out is collected on a filter free from ash, washed with hot and cold water, dried, and incinerated as usual (see above). The resulting weight, multiplied by 1.6 indicates the corresponding amount of oxalic acid in grammes.

LITERATURE.—P. Fürbringer, "Zur Oxalsäureausscheidung durch d. Harn," Deutsch. Arch. f. klin. Med., 1876, vol. xviii. p. 143. J. C. Dunlop, "The Elimination of Oxalic Acid in the Urine," etc., Jour. Path. and Bact., 1896 (an historical review of the subject of oxaluria is here also given). H. Baldwin, "An Experimental Study of Oxaluria," Jour. Exper. Med., vol. v. p. 27. E. Salkowski, Berlin. klin. Woch., 1900, p. 434; and Zeit. f. physiol. Chem., vol. xxix. p. 437. E. Harnack, "Ueber Indicanurie in Folge von Oxalsäurewirkung," Zeit. f. physiol. Chem., 1900, vol. xxix. p. 205.

ALBUMINS.

The albumins which may be met with in the urine are serum-albumin, serum-globulin, albumoses (peptones), the albumin of Bence Jones, hæmoglobin, nucleo-albumin, fibrin, histon, and nucleo-histon. Of these, serum-albumin is the most important from a clinical standpoint.

Serum-albumin.—The question whether or not serum-albumin occurs normally in the urine—*i. e.*, under strictly physiological conditions—has been much disputed. It is claimed by some that traces may be temporarily met with in apparently healthy individuals after severe muscular exercise, cold baths, mental labor, severe emotions, during menstruation, digestion, etc. This so-called *physiological albuminuria* mostly occurs in young adults, and is usually, if not always, of brief duration. The urine, it is claimed, is otherwise normal—*i. e.*, of normal amount, appearance, specific gravity, and composition, and free from abnormal morphological constituents, such as casts, red corpuscles, leucocytes, and epithelial cells.[1]

The existence of a physiological albuminuria, on the other hand, is denied, and the occurrence of serum-albumin at least regarded as pathological in every case. I have never been able to convince myself of the occurrence of serum-albumin in the urine under strictly physiological conditions, and it has been pointed out elsewhere that severe muscular and mental labor, severe mental emotions, cold baths, etc., can hardly be regarded as physiological stimuli for all persons. The albuminuria, so often observed during the first days of life, at which time sediments of uric acid and urates, mucus, epithelial cells from the different portions of the urinary tract, and

[1] C. E. Simon, "Functional Albuminuria," N. Y. Med. Jour., 1895, p. 330.

even casts may also be seen—*i. e.*, constituents which in adults would rightly be regarded as abnormal—has also been brought forward in support of the theory of a physiological albuminuria. There can be no doubt, however, that this form of albuminuria is referable to the profound changes that take place in the circulatory system after birth, and to some extent perhaps also to the well-known uric-acid infarctions so frequently seen in the kidneys of the newly born, so that it would probably be better and more in accord with the teachings of pathology to regard this form of albuminuria also as abnormal.[1]

The more closely the subject of the so-called physiological albuminuria is studied the more improbable does its physiological nature appear, and a more detailed study of the metabolic processes, it may be confidently asserted, will ultimately lead to the conclusion that *the presence of albumin in every case is a pathological phenomenon.*

The association of an increased elimination of urea and uric acid with albuminuria in apparently healthy individuals was noted twenty-five years ago, but received comparatively little attention. More recently, Da Costa[2] has pointed out the existence of albuminuria associated with lithuria and oxaluria. Personal observations have led me to look upon this form of albuminuria as of common occurrence, and while in almost every case the albumin can be caused to disappear from the urine by proper diet and exercise, there can be no doubt that, if neglected, granular atrophy may ultimately result.

An albuminuria may at times be observed in anæmic children and adolescents, and particularly in masturbating boys of the mouth-breathing type, but can hardly be regarded as physiological. The same may be said of the albuminuria of pregnancy and parturition.

As regards the action of cold baths, Rem-Picci[3] reports that albuminuria may be considered a constant phenomenon after cold baths, but that different subjects react differently under the same conditions. Those which show albuminuria more readily are, as a rule, the less robust and thinner individuals, such as are most sensitive to cold. The limits of temperature necessary to produce the phenomenon are from 12° to 13° C., when the immersion is not longer than three minutes. If the temperature be from 15° to 20° C., the albumin appears only after fifteen minutes' immersion. Above this temperature albuminuria does not occur, even if the bath lasts much longer. The colder the bath the more rapid the appearance of albumin. The degree of albuminuria is always slight, and even in the more marked cases rarely exceeds 0.25 pro mille. The sediment, according to Rem-Picci, occasionally shows a few hyaline casts, and often crystals of calcium oxalate.

[1] L. Landi, L'albuminuria nel parto, Morgagni, 1890, vol. xxxii.

[2] Da Costa, "The Albuminuria and Bright's Disease of Uric Acid and Oxalic Acid," Am. Jour. Med. Sci., 1895.

[3] Rem-Picci, "On Albuminuria after Cold Baths," Il Policlinico, 1901, vol. viii. p. 389.

The course which may be taken by these various forms of what should be termed *functional* albuminuria, in which the amount of albumin rarely exceeds 0.1 per cent., is very interesting. The elimination of albumin may thus be quite *transitory* on the one hand, as when following severe muscular exercise, cold baths, and the like. It may, however, also last for several days, or even weeks, and be followed by a disappearance of the albumin for a variable length of time, and again by its reappearance and continuance for days and weeks. The term *intermittent albuminuria*[1] has been applied to this latter type. At times the albuminuria may follow a definite course, disappearing and reappearing with such regularity that it has not improperly been styled *cyclic albuminuria*.[2] In this form the albumin generally disappears from the urine during the night or during prolonged rest in bed, and reappears during the day, the erect posture apparently favoring its reappearance; the term *postural* or *orthostatic albuminuria* has hence also been suggested for this form. Oswald, who made a careful study of cyclic albuminuria in Riegel's clinic, regards its occurrence as distinctly pathological, and as indicating the existence of nephritis. Remembering the importance of the subject, it may not be out of place to enumerate the reasons which led Oswald[3] to this conclusion:

1. The patients generally come to the physician complaining of certain definite symptoms which are similar to those noted in cases of true nephritis. At times, however, no complaints are made, because the patients have reasons for concealing them (as in examinations for life-insurance), or because they are temporarily absent.

2. The subjective complaints, as well as the anæmia so frequently observed in such cases, generally disappear, together with the albumin, under suitable treatment, and reappear when the anæmia again becomes marked.

3. In many, a history of an antecedent nephritis the result of scarlatina or diphtheria may be obtained, as in three cases of Heubner, in fourteen cases out of twenty described by Johnson, etc. In some also a direct transition from an acute nephritis to the cyclic form of albuminuria has been noted. Where this was not possible the history of an acute infectious disease or an angina that had been overlooked in the clinical history must be regarded as a possible cause.

4. The absence of morphological elements, especially tube-casts, does not exclude a nephritis. A large number of cases, moreover, have recently been observed in which casts were repeatedly found.

5. A cyclic albuminuria may be observed in many cases of chronic nephritis.

[1] Bull, Berlin. klin. Woch., 1886, vol. xxiii. p. 717. Mareau, Rev. de méd., 1886, vol. vi. p. 855. Klemperer, Zeit. f. klin. Med., 1887, vol. xii. p. 168.

[2] A. Keller, Beiträge z. Kenntniss d. cyklischen Albuminurie, Diss., Breslau, 1896.

[3] K. Oswald, "Cyklische Albuminurie u. Nephritis," Zeit. f. klin. Med., vol. xxvii. p. 73.

6. Marked organic abnormalities (such as heart-lesions) need not be demonstrable, as they may be absent for a long period of time or may be unrecognizable.

Senator's[1] statement, that the existence of a physiological albuminuria is proved by the fact that the morphological constituents of the primitive nubecula contain albumin, requires no further criticism, and should be regarded as a misconstruction of the main point at issue—a mere sophism; and Posner's[2] observations, in view of the researches of Malfatti,[3] which tend to show that the body obtained by Posner was not serum-albumin, but a nucleo-albumin, may now be regarded as erroneous.

In conclusion, it may be safely asserted that a transitory, intermittent, and cyclic albuminuria is not infrequently observed in apparently healthy individuals, but that the facts so far brought forward do not warrant the assumption that such forms of albuminuria are physiological.[4] The occurrence of such albuminuria unquestionably demonstrates a certain insufficiency of the renal epithelium, and I am much in favor, as Martius has proposed, of discarding the term *physiological* albuminuria altogether, and to speak of these various forms collectively as *constitutional* albuminuria.

It would lead too far to enter into a detailed consideration of the various causes that have from time to time been suggested as an explanation of the fact that albumin does not occur in the urine under normal conditions. There can be no doubt, however, that the integrity of the epithelial lining of the glomeruli and the convoluted tubules must be regarded as the principal factor which prevents the albumin of the blood from passing into the urine. When the readiness with which the glandular structures of the kidney respond to any abnormal stimulation is considered, it is easily understood how an albuminuria may be produced in many different ways. Aside from acute and chronic inflammatory processes in the widest sense of the word, an albuminuria may be the result of circulatory disturbances in the kidneys of whatever kind—*i. e.*, the result of anæmia as well as of hyperæmia. In many and perhaps the majority of cases in which what Bamberger[5] terms a *hæmatogenous albuminuria* occurs, we have direct evidence of the existence of circulatory disturbances, as in cases of uncompensated valvular lesions, weak heart, emphysema, hepatic cirrhosis, etc. In other cases, however, the existence of such disturbance can only be surmised, and the

[1] Senator, Die Albuminurie, Hirschwald, Berlin, 1882.

[2] C. Posner, Berlin. klin. Woch., 1885, vol. xxii. p. 654; Virchow's Archiv, 1886, vol. civ. p. 497; Arch. f. Anat. u. Physiol., 1888.

[3] Malfatti, Internat. Centralbl. f. d. Physiol. u. Pathol. d. Harn- u. Sexualorgane, 1889, vol. i. p. 266.

[4] v. Noorden, Deutsch. Arch. f. klin. Med., vol. xxxviii. pp. 3 and 205. Leube, Zeit. f. klin. Med., 1887, vol. xiii. p. 1. Winternitz, Zeit. f. physiol. Chem., 1891, vol. xv. p. 189. C. E. Simon, loc. cit.

[5] v. Bamberger, Wien. med. Woch., 1881, pp. 145 and 177.

question, whether or not the albuminuria observed in the various infectious diseases, for example, is referable to circulatory abnormalities or to a direct irritative action of microbic poisons upon the renal parenchyma, must still remain open.

From personal studies in connection with the functional albuminuria of Da Costa, it seems not unlikely that in many cases in which obscure circulatory disturbances are supposed to exist and are held responsible for an existing albuminuria, this is referable rather to the strain thrown upon the kidneys by the continued elimination of abnormally large quantities of organic material, the quantity of water being at the same time proportionately small.

If it is remembered, furthermore, that injuries affecting certain portions of the brain are followed by albuminuria, and that this may be artificially produced by a *piqure*, analogous to the glucosuric *piqure* of C. Bernard, still another factor is given which may possibly enter into the causation of albuminuria.

Obstruction to the outflow of urine from the kidneys has also been experimentally shown to lead to albuminuria, an observation with which clinical experience is in perfect accord.

In patients actually in labor albuminuria is common, and supposedly due to increased blood-pressure in the kidneys caused by uterine contractions and the general disturbance of the circulation. The relative frequency of its occurrence is a matter of dispute, however, and widely differing statements are made by different observers, ranging from 15 to 20 per cent. (Petit, Winckel) to 99 and 100 per cent. (Trautenroth, Pajikull).

As regards the occurrence of albuminuria in pregnancy the results of different observers likewise differ, viz., from 1 to 50 per cent. In the last months of pregnancy Zangemeister[1] found albumin in 10 per cent. of the cases examined, and if repeated examinations were made positive results were obtained persistently during the last three months in 40 per cent. The albuminuria is supposedly referable to some metabolic disturbance and impaired excretion by the kidneys.

Finally, an abnormal composition of the blood may at times cause the albuminuria.

In passing on to a more detailed study of the various pathological conditions in which an elimination of albumin may be noted, an attempt will be made to classify the various forms of albuminuria in accordance with the more general considerations set forth above. It should be remembered, however, as already indicated, that it may be very difficult, if not impossible, to assign one single cause to a given clinical case, as several factors may at the same time be operative in the production of the albuminuria.

1. Functional Albuminuria.—Under this heading may be comprised the various forms of "physiological" albuminuria, which have already been considered.

[1] Zangemeister, Arch. f. Gyn., 1902, vol. lxvi. Heft 2.

2. The albuminuria associated with organic diseases of the kidneys, viz., acute and chronic nephritis, renal arteriosclerosis, amyloid degeneration of the kidneys.[1]

In acute nephritis, albuminuria, usually of great intensity, is a constant and most important symptom. The amount eliminated is generally proportionate to the intensity of the disease, but varies within fairly wide limits, generally from 0.3 to 1 per cent., corresponding to a daily excretion of from 5 to 8 grammes. Much larger quantities, it is true, are at times excreted, but it may be definitely stated that the daily loss of albumin seldom exceeds 20 grammes.

In chronic parenchymatous nephritis the elimination of albumin is likewise constant, and the amount excreted in severe cases may even exceed that observed in the acute form. An elimination of from 15 to 30 grammes, viz., 1.5 to 3 per cent. by weight, is frequently observed.

In the ordinary form of chronic interstitial nephritis the elimination of albumin is, as a general rule, slight, and rarely amounts to more than 2 to 5 grammes pro die. At the same time it is not unusual to meet with an apparent absence of albumin if the more common tests (see below) are employed. If it is remembered that very often the diagnosis of the disease is dependent upon the demonstration of the presence or absence of albumin, the necessity of *frequent* examinations and the employment of more delicate tests, particularly of the trichloracetic acid test, as well as of a microscopical examination, is at once apparent. This is even of greater importance in the renal arteriosclerosis of Senator, in which albumin by the ordinary tests is probably not demonstrable in the majority of cases, and in which even the trichloracetic acid test *may* not be of service, and casts are absent.

Amyloid degeneration of the kidneys, in the absence of inflammatory processes, is accompanied by a condition of the urine closely resembling that observed in the ordinary form of chronic interstitial nephritis. A total absence of albumin, however, is less frequently noted, while an amount varying between 1 and 2 per cent. is not uncommon. It will be shown later on that in this condition considerable amounts of serum-globulin are excreted in addition to the serum-albumin; larger amounts, in fact, than are generally observed in this form of chronic renal disease, so that Senator suggests that such a relation, in the absence of an acute nephritis, or an acute exacerbation of a chronic nephritis, may be of a certain diagnostic value.

3. Febrile Albuminuria.[2]—That albuminuria may occur in almost any one of the various febrile diseases is a well-known fact,

[1] Senator, loc. cit.

[2] Leyden, Zeit. f. klin. Med., 1881, vol. iii. p. 161. H. Lorenz, Wien. klin. Woch., 1888, vol. i. p. 119.

but it is important to remember that, while such an albuminuria *may* at times be referable to a true nephritis developing in the course of or during convalescence from an acute febrile disease, such is the exception, and not the rule. Under this heading, only that form will be considered which is not associated with distinct changes affecting the renal parenchyma, and which generally appears during the height of the disease only, and disappears with a return of the temperature to normal. As has been mentioned, it is often difficult, if not impossible, to assign a definite cause for an albuminuria of this character, and in all probability several factors are in operation at the same time. In the beginning of the disease, when the blood-pressure, as a rule, is increased, the albuminuria may be referable to an ischæmia of the kidneys, as the increased pressure in fever, according to Cohnheim and Mendelson, is largely referable to spasm of the arterioles. Later on, or in the beginning of cases in which especially severe intoxication exists, the blood-pressure may be subnormal, and the albuminuria be due to this cause—*i. e.*, a hyperæmic condition of the kidneys. As a matter of fact, it has been experimentally demonstrated that both anæmia and hyperæmia of the kidney structure may lead to albuminuria. On the other hand, it is not unlikely that the strain thrown upon the kidneys by an excessive elimination of organic material, in the absence of a correspondingly large quantity of water, may produce albuminuria. I have repeatedly seen the functional albuminuria of the type described by Da Costa disappear during the administration of a diet relatively poor in nitrogen, while an increased diuresis was at the same time effected by the consumption of large amounts of water.

In those grave cases of typhoid fever, furthermore, which are characterized by high fever and pronounced nervous symptoms it would appear quite likely that the albuminuria, which in these cases is particularly marked, is referable to a direct influence upon the central nervous system, and in some cases, at least, also dependent upon an irritant action upon the renal epithelium on the part of the microbic poisons circulating in the blood. The character of the albuminuria will largely depend upon the intensity of the intoxication; in other words, upon the amount of bacterial poison present at any one time in the blood.

Notwithstanding statements to the contrary, albuminuria may be regarded as a constant symptom of typhoid fever, as has been definitely demonstrated by Gubler and Robin. It is difficult to say why other observers have found albumin in only a comparatively small percentage of cases, but it is not unlikely that this is owing to a lack of uniformity in methods, it being presupposed also that questions of this kind can only be decided by *daily* examinations. According to Robin, the trace of albumin which is at times observed during

the first week of the disease is an albumose, while later on serum-albumin is constantly found; the amount increases with the intensity of the morbid process, and the highest figures are reached in fatal cases. The more severe the disease the earlier does albumin appear in the urine, it being remembered, however, that reference is had only to those cases in which distinct renal changes are not demonstrable. Toward the termination of the fastigium the amount of albumin generally undergoes a certain diminution, and may even disappear entirely. This diminution, however, is only temporary, and in severe cases the albumin again increases in amount during the period of great variations in the temperature. In light cases an increased elimination also takes place at this stage, but is soon followed by a decrease, after which only traces can be demonstrated. In some cases it disappears entirely, but it is rare, according to Robin, to meet with cases in which at least a trace does not reappear during convalescence.

In light cases the albuminuria rarely persists longer than the fifth or eighth day of convalescence, and Robin even goes so far as to say that a relapse may be anticipated if the albuminuria does not disappear at that time. A limited number of personal observations have borne out the correctness of this view, and in one case in which a relapse occurred so late as the fifteenth day of convalescence traces of albumin could be demonstrated during the entire period. In severe cases, on the other hand, the albumin persists for a variable length of time, and rarely disappears before the tenth day of convalescence. At times an increase is seen during convalescence when traces only have previously been observed. It is this form which the French generally speak of as *colliquative albuminuria.* While this is principally observed in typhoid fever, it is not unusual to meet with it during convalescence from various other acute diseases. Care must be taken not to confound the albuminuria so frequently seen during convalescence from typhoid fever, referable to a pyelitis, with the form just described.

From the following summary, constructed from data given in Robin's[1] monograph on the urine of typhoid fever and other acute infectious diseases which may be associated with a typhoid condition, an idea may be formed of the occurrence of albuminuria, as well as of its degree of intensity in these diseases:

Acute miliary tuberculosis: albumin is much less frequent than in typhoid fever; when present, it is rarely found in the abundance so characteristic of the fatal cases of the latter disease.

Pneumonia: albumin is as uniformly present as in typhoid fever, and at times very abundant.

Grippe: albumin is infrequent; present in about 20 per cent. of the cases, and only in traces.

[1] A. Robin, Urologie clinique de la fièvre typhoide, Paris, 1877.

Herpetic fever: albumin never present in large amounts.

Embarras gastrique: albumin rarely present.

Adynamic enteritis of adults: albumin almost always present, but usually only in traces.

Cerebrospinal meningitis: albumin in fairly large amounts.

Vegetative endocarditis: albumin very abundant in about 14 per cent., evident in 44 per cent., and traces in 42 per cent. of all cases.

Acute articular rheumatism: albumin present in about 40 per cent.

Rubeola: albumin usually absent in light cases, but present in the more severe and complicated forms.

Intermittent fever: albumin variable.

In a series of 799 cases of pneumonia reported from the Boston City Hospital,[1] albumin was found in 624—*i. e.*, in 78 per cent. It was noted that the death-rate bore a direct ratio to the amount of albumin in the urine.

In smallpox a trace of albumin is practically constant. Somewhat larger amounts are found in about 30 per cent. of all cases. The albuminuria is most marked during the eruptive stage and then rapidly diminishes in intensity. More rarely it reaches its maximum during the suppurative fever stage, or during convalescence.[2]

As the result of the examination of a large number of cases of plague Corthorn[3] arrived at the conclusion that no albumin is found in only 14 per cent. of all cases. In cases ending in recovery the albuminuria never occurred later than the fourth day.

In conclusion, it may be said that practically every acute febrile disease, even simple follicular tonsillitis, may be accompanied by albuminuria in the absence of definite changes affecting the renal parenchyma. Its occurrence in an individual case is probably dependent to a very large degree upon the intensity of the intoxication. While it is generally an easy matter to distinguish between this form of albuminuria and that associated with distinct organic changes in the kidneys, considerable difficulty may at times be experienced; this question will be dealt with later on.

4. Albuminuria referable to Circulatory Disturbances.[4]—To this class belongs the albuminuria so frequently observed in cardiac insufficiency referable to valvular lesions, degeneration of the heart-muscle from whatever cause, disease of the coronary arteries, etc., as well as in cases of impeded pulmonary circulation affecting the general circulation through the right heart, and, finally, in conditions associated with local circulatory disturbances, such as com-

[1] Sears and Larrabee, Med. and Surg. Rep. of the Boston City Hospital, 12th Series, Dec., 1901.

[2] Arnaud, "Albuminurie et lesions des reins dans la variole," Rev. d. Méd., 1898, vol. xviii. p. 392.

[3] Corthorn, "Albuminuria in Plague," Brit. Med. Jour., Sept. 14, 1901.

[4] Senator, loc. cit.

pression of the renal veins by a pregnant uterus, tumors, etc. It has been pointed out that febrile albuminuria also may, to a certain extent at least, be referable to such causes—*i. e.*, an ischæmia or hyperæmia of the kidneys produced by an increased or diminished blood-pressure. The albuminuria observed in cases of cholera infantum, the simpler forms of intestinal catarrh, and in cholera Asiatica particularly, are undoubtedly dependent upon such causes. The occurrence of albuminuria after cold baths, as stated above, is regarded by many as a "physiological" phenomenon; but this view should be rejected, as there can be little doubt that this form is also referable to circulatory disturbances. The quantity of albumin found under these circumstances varies considerably, but rarely exceeds 0.1–0.2 per cent. unless the disease has advanced to a stage where distinct changes in the renal parenchyma have resulted.

5. Albuminuria referable to an Impeded Outflow of Urine.—Clinically, albuminuria referable primarily to an impeded outflow of urine from the kidneys is probably of more frequent occurrence than is generally supposed, and especially in women, in whom Kelly and others have demonstrated the frequent existence of ureteral stenoses. A complete blocking of the excretory duct, on the other hand, is rarely seen, but may be caused by the impaction of a renal calculus, the pressure of a tumor, or following certain gynæcological operations in which the ureter is accidentally caught in a suture, etc. It has also been suggested that the albuminuria of pregnancy may be due to compression of a ureter, but it is more likely that other factors are here at play, such as compression of the renal arteries and veins.

6. Albuminuria of Hæmic Origin.[1]—It was formerly supposed that Bright's disease was dependent upon certain abnormalities of the blood, and as a matter of fact this view has not only never been disproved, but is actually gaining ground from day to day. According to Semmola, Bright's disease is primarily due to an abnormal power of diffusion on the part of the albumins of the blood, which are eliminated by the kidneys as waste material. As a result of the excessive amount of work thus done definite renal changes are finally produced. According to his theory, then, the albuminuria is the primary factor in the causation of nephritis. Should this hypothesis hold good, Senator is correct in asserting that an albuminuria of functional origin, so to speak, must precede the occurrence of the nephritis proper. He, however, doubts the occurrence of a prenephritic albuminuria; but others have noted the occurrence of definite renal changes which manifestly followed an apparently functional albuminuria (Da Costa). Further researches in this direction are urgently needed, and Semmola's view can at present only be regarded as an hypothesis. But even if such blood-changes as

[1] v. Bamberger, loc. cit.

those which Semmola suggests should not exist, there can be little doubt that true nephritis is dependent upon an acute or chronic dyscrasia of the blood, either in the sense of an abnormal mixture of the normal elements or of the presence of abnormal constituents, and notably of poisons. The same considerations undoubtedly also apply to various other forms of albuminuria, in so far as these are not the direct result of circulatory disturbances.

Clinically, albuminuria of hæmic origin is observed in various diseases of the blood, such as purpura, scurvy, leukæmia, pernicious anæmia, as also in cases of poisoning with lead and mercury, in syphilis, jaundice, diabetes, following the inhalation of ether and chloroform, etc. The albuminuria associated with an excessive elimination of uric acid and oxalic acid, and, according to personal observations, with an excessive elimination of organic material in general, notably of urea; probably also belongs to this class.

7. Toxic Albuminuria.—It has already been stated that the albuminuria of acute febrile diseases may, to a certain extent, be referable to a direct irritant action on the part of bacterial poisons upon the renal parenchyma. Poisoning with cantharides, mustard, oil of turpentine, potassium nitrate, carbolic acid, salicylic acid, tar, iodine, petroleum, phosphorus, arsenic, lead, antimony, alcohol, and mineral acids produces albuminuria. In all probability, however, the albuminuria here observed is referable not only to a direct irritant action upon the glandular epithelium of the kidneys, but also to circulatory disturbances.

8. Neurotic Albuminuria.—It is claimed by some that albumin, usually in small amounts, is eliminated in epilepsy after every attack, while others either deny its occurrence under such conditions or regard it as exceptional. In a number of cases in which I had occasion to examine urine voided after an attack albumin was usually absent. It should be stated, however, that the seizures in these cases were comparatively slight, and that unfortunately an examination for semen was not made in those urines in which traces of albumin were demonstrated. An examination of the urine voided by a patient, after having been in the epileptic state for more than forty-eight hours, showed the presence of a small amount of albumin associated with an enormous elimination of uric acid, as well as a large excess of urea. Semen was absent.[1] Nothnagel states that he could not demonstrate any regularity in the appearance of albumin. In some of his cases with major attacks there was no albumin; in others it appeared after every attack; in still others it was sometimes present and at other times absent (in the same individual). At times it was found after a minor attack and was absent after a major attack (also in the same individual).

Other observers have obtained similar results, so that we may

[1] M. Huppert, Virchow's Archiv, 1874, vol. lix. p. 305.

conclude that albuminuria following epileptic seizures is rather the exception than the rule. When it does occur, its significance is essentially the expression of a certain grade of cyanosis during the attacks.[1]

A transient albuminuria has also been noted in cases of progressive paralysis, mania, tetanus, delirium tremens, apoplexy, migraine, Basedow's disease, brain tumor, etc.

Although albuminuria may apparently be produced artificially by injuries affecting a certain area in the floor of the fourth ventricle, analogous to the production of glucosuria (see Glucosuria), it would probably be going too far to assume the existence of a certain specific centre, stimulation of which causes the appearance of albumin in the urine. While the influence of the nervous system in preventing the passage of albumin through the glomeruli under normal conditions is undoubted, it would appear more likely that the albuminuria following injuries to the central nervous system is referable to circulatory disturbances in the kidneys secondary to lesions of the brain, and especially of the medulla. The albuminuria observed in certain neurotic individuals, on the other hand, is probably more frequently associated with metabolic abnormalities, and is of hæmic origin.

9. A DIGESTIVE ALBUMINURIA has also been described.[2] It may follow the ingestion of excessive amounts of cheese, eggs—particularly when taken raw—beef, etc. Specially interesting is the form which follows the ingestion of excessive amounts of egg albumin. Ordinarily the consumption of a moderate amount of such albumin does not lead to albuminuria, while in cases of nephritis an already existing albuminuria is increased. But it has also been noted that even in individuals with *apparently* healthy kidneys, the ingestion of an excessive amount of egg albumin may call forth albuminuria, and it is possible in both cases to demonstrate the presence in the urine of both egg albumin and blood albumin.

To examine into this question the individual is given from four to eight raw eggs on an empty stomach in the morning for two to four days. His diet otherwise is as usual. The urine is collected in intervals of from two to three hours. If the ingestion of such an amount of egg albumin leads to albuminuria, this usually occurs after about four hours, and reaches its maximum intensity two hours later. Casts are not found (Jnouye).

The albuminuria in question, so far as the egg albuminuria goes, is undoubtedly owing to the fact that a certain amount of egg albumin is absorbed as such from the gastro-intestinal canal and is subsequently eliminated as foreign material. In what manner, however,

[1] Nothnagel, Ziemssen's Handbuch, 1877, vol. xii. p. 179. Binswanger, Nothnagel's spec. Pathol. u. Therap., vol. xii. p. 235 (literature).

[2] Ascoli, "Ueber d. Mechanismus d. Albuminurie durch Eiereiweiss," Münch. med. Woch., 1902, No. 10. Jnouye, "Ueber alimentäre Albuminurie," Deutsch. Arch. f. klin. Med., 1902, vol. lxxv. p. 378.

the egg albuminuria may be responsible for the accompanying serum albuminuria is more difficult to explain.

Of the albuminuria which follows excessive indulgence in cheese and beef but little is known. Bearing in mind that the albuminuria very often follows the ingestion of such articles almost immediately, and before they have become absorbed, it is hardly justifiable to refer this form to the existence of a hyperalbuminosis. It would appear more rational, as Senator has suggested, to think of reflex vasomotor or trophic changes affecting the kidneys; while in other cases, in which the albuminuria does not follow the ingestion of such articles of food immediately, it is quite probable that is may be dependent upon certain metabolic abnormalities affecting the normal composition of the blood.

In the account thus given of the occurrence of albuminuria and its possible causes, reference has been had to only a *purely renal* albuminuria. It should be remembered, however, that the origin of the albumin may often be extremely difficult to determine, as albuminous material, such as blood and pus, may become mixed beyond the glandular portion of the kidneys with what would otherwise have been a perfectly normal urine, and that such an admixture may take place not only in the ureters, the bladder, and the urethra, but even in the pelvis of the kidney.

The term *accidental albuminuria* is applied to a condition in which albuminous material becomes mixed with a urine beyond the kidneys, as in cases of cystitis and urethritis, or whenever semen has entered the urine while the renal urine proper is free from albumin. An admixture of pus, blood, lymph, or chyle may, however, also occur in the kidneys, when the albuminuria is termed *accidental renal albuminuria*, an example of which is frequently seen in the slight degree of albuminuria referable to pyelitis during convalescence from typhoid fever. By a *mixed albuminuria* and a *mixed renal albuminuria*, on the other hand, we are to understand conditions in which the source of the albumin is twofold, renal and extrarenal in the first instance, parenchymal and extraparenchymal in the second, examples being the albuminuria of cystitis combined with nephritis and pyelonephritis, respectively.

It is manifest, of course, that in every instance in which albumin is found in the urine its origin should be ascertained. While this question is usually readily decided by a microscopical examination of the urine, considerable difficulty may occasionally be experienced. It is a well-known fact that in the urine of women a trace of albumin may frequently be detected, which is not due to any lesion of the urinary organs, but to an admixture of vaginal discharge, of blood during the process of menstruation, and, in married women, of semen. Whenever, therefore, doubt is felt as to the origin of the albumin, the specimen for examination should be obtained by the

catheter, care being taken previously to cleanse the vulva. In men albumin may be referable to a gonorrhœal urethritis. In such cases it is well to let the patient flush out his urethra first, and to make use for examination of the portion last voided. Very often, however, the conditions are more complex, it being uncertain whether the albumin is referable to the presence of pus only, or whether its origin is in the renal parenchyma. In such cases, as in cystitis, pyelonephritis, etc., a careful microscopical examination and enumeration of the pus-corpuscles with the Thoma-Zeiss instrument are called for, and will in the majority of instances decide the question. Generally speaking, the amount of albumin found in uncomplicated cases of cystitis does not exceed 0.15 per cent., while in cases of pyelitis of the same intensity the amount of albumin is from two to three times as large.

Of late, attention has repeatedly been drawn to the occasional presence in the urine of an albuminous body which is soluble in acetic acid, and which Patein regards as a modification of common serum-albumin. It has thus far been observed in only eight cases, viz., twice in chronic nephritis, three times in eclampsia, once in a cystic kidney, once in tonsillitis following an injection of diphtheria antitoxin, and once in a pregnant woman in whom typhoid fever developed. I should suggest that the substance be spoken of as *Patein's albumin*[1] until its chemical identity has been established. The term *aceto-soluble albumin* is, of course, likewise admissible.

So far as the *amount of albumin* which may be eliminated in the twenty-four hours is concerned, an excretion of less than 2 grammes may be regarded as insignificant, 6 to 8 grammes as a moderate amount, and 10 to 12 grammes or more as excessive. An excretion of 20 to 30 grammes is exceptional.

Serum-globulin.—It has been pointed out that in cases of amyloid degeneration of the kidneys serum-globulin is found in the urine together with serum-albumin in large amounts, and, according to Senator, a ratio between the two albumins of 1 : 0.8 : 1.4 may be regarded as a fairly constant symptom of the disease, and is of diagnostic importance. There seems to be no doubt, however, that serum-globulin occurs in the urine, although in much smaller quantities than in the disease mentioned, whenever serum-albumin is eliminated.[2]

A most remarkable instance of globulinuria has been recorded by Noel Paton,[3] in which the globulin separated out in crystalline form and was found in extraordinarily large quantity, amounting on one day to 70 grammes.

[1] Patein, "Aceto-soluble Albumin in the Urine," Compt. rend. de l'Acad. des Sci., 1889. Coplin, Phila. Med. Jour., 1899, p. 957.

[2] Edlefsen, Deutsch. Arch. f. klin. Med., vol. vii. p. 67. Senator, Virchow's Archiv, vol. lx. p. 476. Petri, Diss., Berlin, 1876.

[3] B. Bramwell and N. Paton, Laboratory Reports of the Royal College of Physicians, Edinburgh, 1892, vol. iv. p. 47.

Albumoses.—Albumoses have frequently been encountered in the urine, but are probably more frequently overlooked, as the bodies in question are not precipitated on boiling. In former years they were commonly regarded as peptones. At present, however, it appears to be a well-established fact that true peptones, in the sense of Kühne, viz., true albumins which are not precipitated by salting with ammonium sulphate, do not occur in the urine, and the term peptonuria should accordingly be abandoned.

Albumosuria is observed under a great variety of conditions. It is thus noted in association with large accumulations of pus within the body, and there can be little doubt that the albumosuria is in such instances referable to a disintegration of the pus-corpuscles and a resorption of the resulting albumoses. This form has hence been termed *pyogenic albumosuria.* It is principally observed during the stage of resolution in cases of croupous pneumonia; in association with pyothorax, and in cases of epidemic cerebrospinal meningitis, as contrasted with the tubercular form. A *hepatogenic form* is noted in connection with diseases of the liver, notably acute yellow atrophy. Of its origin, however, nothing is known. Formerly, when the condition was looked upon as a peptonuria, and when it was thought that peptones were retransformed into native albumins in the liver, the "peptonuria" was explained upon the assumption that the liver had lost its power, and that the "peptones" accumulated in the blood, and were consequently eliminated in the urine. Later researches showed that the transformation of peptones into albumins takes place in the intestinal mucous membrane, and that the liver probably has no part in the process whatsoever. The explanation given had therefore to be abandoned, and, as I have just indicated, we know nothing whatever of the origin of this hepatic albumosuria. Possibly it is of an enzymatic nature.

An *enterogenic form* of albumosuria has been noted in various diseases of the intestinal tract, such as typhoid fever, tubercular ulceration, carcinoma, etc.; and it is possible that in these cases the albumoses are either directly absorbed from disintegrating pus, or that the intestine perhaps has in part lost the power of preventing the resorption of albumoses as such into the blood.

A *histogenic* or *hæmatogenic* origin has been ascribed to the albumosuria which is seen in cases of scurvy, in dermatitis, in various forms of poisoning, during the puerperal period and pregnancy, particularly following death of the fœtus, in various psychoses, in cases of carcinomatosis, acute yellow atrophy, etc.

A *renal* or *vesical form* of albumosuria is further noted in which the albumoses are derived from contained albumins, owing either to the presence of the common proteolytic ferments of the urine or to bacterial action, as in decomposing albuminous urines.

Aside from the conditions already mentioned, albumosuria has been observed in various infectious diseases, such as septicæmia, pyæmia, diphtheria, measles, scarlatina, acute articular rheumatism, mumps, malaria, phthisis; further, in association with leukæmia, nephritis, puerperal parametritis, endocarditis, caries, pleurisy, heart disease, apoplexy, myxœdema, carcinomatous peritonitis, in pneumonia at the height of the disease and before resolution has set in, in liver abscess, etc.

In the differential diagnosis of suppurative meningitis a positive peptone-reaction in the older sense of the word, according to Senator, speaks strongly in favor of the existence of this disease. In support of this view he cites the case of a young man, the subject of a median otitis of long standing, in which symptoms pointing to a meningitis—viz., fever, headache, and pains in the neck—were present, but in which no "peptonuria" was found to exist, and in which an operation revealed the presence of a cholesteatoma.

A *digestive form* of albumosuria has recently been described, in which albumoses appear in the urine after their ingestion in large quantities, and it is claimed that this is observed only in cases of ulcerative disease of the intestinal tract. Only a positive result, however, is of value.

Very frequently albumosuria accompanies albuminuria, a condition which Senator has termed *mixed albuminuria*, and it is interesting to note that the albumosuria may alternate with the albuminuria, and may precede or follow the latter. In any case in which albumoses can be demonstrated in the urine the appearance of albumin should accordingly be anticipated.

In all cases of albumosuria the amount of albumose that appears in the urine is relatively small, and as a rule cannot be demonstrated by the biruret test when applied directly to the native urine. On the contrary, it is necessary to isolate the substance more or less definitely before deductions can be drawn as to its presence or absence.

LITERATURE.—Hofmeister, Prag. med. Woch., 1889, vol. v. pp. 321 and 325. v. Noorden, Lehrbuch d. Path. d. Stoffwechsels, Hirschwald, Berlin, 1893, p. 215. Senator, Deutsch. med. Woch., 1895, vol. xxi. p. 217. Stadelmann, Untersuchungen über Peptonurie, Bergmann, Wiesbaden, 1894. v. Jaksch. Prag. med. Woch., vol. v. pp. 292 and 303, and vol. vi. pp. 61, 74, 86, 133, 143; Zeit. f. klin. Med., 1883, vol. vi. p. 413. Krehl u. Matthes, Arch. f. klin. Med., 1895, vol. xlv. p. 54. Maixner, Zeit. f. klin. Med., 1884, vol. viii. p. 234. Fischel, Arch. f. Gynaek., 1884, vol. xxiv. p. 27. v. Jaksch, Prag. med. Woch., 1895, vol. xx. p. 430. Katz, Wien. med. Blätter, 1890, vol. xiv. L. v. Aldor, Berlin. klin. Woch., 1899, pp. 765 and 785.

Bence Jones' Albumin.—In association with the occurrence of multiple myeloma of the bones, notably when affecting the thoracic skeleton, a peculiar albuminous body is found in the urine, which is apparently pathognomonic of the disease in question. It was first observed by Bence Jones, and has heretofore been regarded as an albumose. From the researches of Magnus Levy and my

own investigations, however, it appears that the substance is in reality a true albumin, as it yields a proto-albumose on peptic digestion; but it differs from all known albumins in its relative solubility on boiling, and in the readiness with which it dissolves in dilute ammonia after precipitation with alcohol. Like caseïn, it contains no hetero-group, but is distinguished from it by the presence of a carbohydrate radicle and the probable absence of phosphorus. It is crystallizable, and may occur in the urinary sediment in the form of typical spheroliths.

The amount of the substance which may be found in the urine is variable. Some observers have noted an elimination of from 0.25 to 6.0 pro mille, while others report much larger quantities. In Bence Jones' case the elimination rose on one occasion to 6.7 per cent., corresponding to a total output of 70 grammes in the twenty-four hours—*i. e.*, to nearly as much as the entire amount of the albumins of the blood-plasma.

As regards the origin of the albumin, nothing definite is known, but there is reason to suppose that it is not derived from the myelomatous tissue as such. We may imagine, however, that through the agency of the cells of the abnormal tissue, viz., their products of metabolism, the normal transformation of the ingested albumins into tissue-albumins is impeded, resulting in the production of the substance in question, which is then eliminated as foreign matter.

The disease seems to be comparatively rare, and thus far only about twenty-eight cases have been reported in which due attention was paid to the condition of the urine. Besides these there are a few additional cases in which no special note was made of this point, though Zahn states that in his case "sometimes more and sometimes less albumin" was found. Runeberg also reports that the urine of his patient contained much albumin, while the kidneys were found practically normal at autopsy.

As the diagnosis of the disease, in its early stages at least, is altogether dependent upon the demonstration of the albumin in question, a special examination should be made in this direction in all cases of obscure bone-pain, as also in obscure cases of anæmia, since Ellinger has shown that at times the disease may take its course without the occurrence of local symptoms, while a marked anæmia may exist.

Of special interest in this connection is the fact that Zülzer claims to have succeeded in bringing about the appearance of Bence Jones' albumin in the urine of animals by feeding with pyrodin, which is known to be a distinct hæmolytic poison.

LITERATURE.—Bence Jones, Med. and Chir. Trans., 1850, vol. xxxiii.; and Phil. Trans. Royal Soc. of London, 1848. Kühne, "Ueber Hemialbumose im Harn," Zeit. f. Biol., vol. xxix. p. 209. Ellinger, "Ueber d. Vorkommen d. Bence Jones'schen Körper im Harn," Arch. f. klin. Med., 1898, vol. lxii. p. 255. Magnus Levy, Zeit. f. physiol. Chem., 1900, vol. xxx. p. 200. Hamburger, Johns Hopkins Hosp. Bull., Feb., 1901. Zülzer, Berlin. klin. Woch., 1900, p. 894. C. E. Simon, Am. Jour. Med. Sci., 1902, vol. cxxiii. p. 954.

Peptonuria.—To judge from recent investigations of Ito,[1] true peptone in the sense of Kühne, may, after all, occur in the urine under pathological conditions. He obtained positive results in pneumonia, in advanced cases of phthisis, in ulcer of the stomach, and in several women after childbirth. The reaction was most intense in the pneumonia cases; it appeared already before resolution occurred, and disappeared a few days after the crisis. In the parturient women no reaction was obtained if the examination was delayed until after the tenth day. It is noteworthy that in the cases examined by Ito the peptonuria was always associated with the presence of albumoses (deutero-albumoses), and that the peptone was present in still smaller amount than the albumoses.

Hæmoglobin (Methæmoglobin).—Under normal conditions the disintegration of the red blood-corpuscles which is constantly taking place in the body never results in such a degree of hæmoglobinæmia as to be followed by an elimination of hæmoglobin in the urine. Whenever the destruction of red corpuscles is so extensive, however, that the liver is unable to transform into bilirubin all the blood-coloring matter set free, *hæmoglobinuria* occurs. While these factors, then—*i. e.*, an excessive destruction of the red blood-corpuscles and an insufficiency on the part of the liver—must be regarded as explaining every case of hæmoglobinuria, our knowledge of the ultimate causes of such excessive disintegration, as well as the manner in which these operate, is limited. Formerly the term *hæmatinuria* was applied to this condition. It was shown, however, that the pigment eliminated is in reality not hæmatin, but usually methæmoglobin, and only at times hæmoglobin, so that the term hæmoglobinuria is also ill chosen.

Most common is the hæmoglobinuria produced by certain poisons, such as potassium chlorate, arsenious hydride, hydrogen sulphide, pyrogallic acid, naphtol, hydrochloric acid, tincture of iodine, carbolic acid, carbon monoxide, etc., and also by morels (Helvella esculenta).

Quite familiar is the hæmoglobinuria observed following transfusion of the blood of animals into man, such as that of the calf and lamb; also the form seen in extensive burns and in insolation.

While hæmoglobinuria may occur in the course of any one of the specific infectious diseases, such as scarlatina, icterus gravis, variola hæmorrhagica, typhoid fever, yellow fever, etc., it is said to be especially frequent in cases of malarial intoxication. This view is not accepted by many; Osler, among others, believes that it has frequently been confounded with malarial hæmaturia. I have never seen an instance of malarial hæmoglobinuria, and believe that in our more temperate zones it scarcely ever occurs. Bastianello asserts that it is likewise rare in Italy, but more common in Sicily

[1] M. Ito, "Ueber d. Vorkommen v. echtem Pepton im Harn," Deutsch. Arch. f. klin. Med., 1901, vol. lxxi. p. 29.

and Greece, and very common in the tropics. According to the same observer, hæmoglobinuria occurs only in infections with the æstivo-autumnal parasite. A hæmoglobinuria due to quinin is likewise said to exist, but is certainly rare, excepting in patients who are suffering or have recently suffered from malarial fever. I have seen but one instance of hæmoglobinuria following the ingestion of quinin. To judge from the literature upon the subject, there can be no doubt that syphilis may under certain conditions be a potent factor in the production of hæmoglobinuria. This appears to be particularly true of those cases of so-called paroxysmal hæmoglobinuria in which bloody urine is voided from time to time, the attacks being frequently preceded by chills and fever, so as closely to simulate malarial fever. Other factors, also, notably cold, appear to be concerned in the production of this form.

The occasional occurrence of hæmoglobinuria in cases of Raynaud's disease, coincident with attacks of an epileptiform character, has been referred to in the chapter on the Blood (see page 36).

Hæmoglobinuria has been observed in a case of leukæmia complicated by icterus.

Finally, an epidemic hæmoglobinuria has been described as occurring in the newborn associated with jaundice, cyanosis, and nervous symptoms; of its causation we are in ignorance.

While hæmoglobinuria is rather uncommon, *hæmaturia* is frequently observed, and will be considered later on, as its recognition is not dependent upon the demonstration of the albuminous body, "hæmoglobin," alone in the urine, but upon the presence of red corpuscles, which in hæmoglobinuria are either absent or present in only very small numbers.

LITERATURE.—Hæmoglobinuria: Rosenbach, Berlin. klin. Woch., 1880, vol. xvii. pp. 132 and 151. Ehrlich, Zeit. f. klin. Med., 1881, vol. iii. p. 383. Boas, Arch. f. klin. Med., 1885, vol. xxxii. p. 355. Kobler u. Obermayer, Zeit. f. klin. Med., 1888, vol. xiii. p. 163.

Fibrin.—The occurrence of fibrin in the urine presupposes the presence of fibrinogen and a fibrinogenic ferment. It is seldom seen. According to Neubauer and Vogel, the fibrin may occur either as coagulated fibrin or in solution. In the former condition it is at times observed in the form of blood-coagula, when its significance is essentially the same as that of hæmaturia in general, although it must be remembered that the usual form of hæmaturia is not associated with the presence of coagula. Colorless coagula of fibrin are seen in cases of chyluria or diphtheritic inflammation of the urinary passages. On the other hand, urines containing fibrinogenic material in solution are likewise seen but rarely, and are characterized by the fact that fibrinous coagula separate out only *on standing*, when they usually cover the bottom of the vessel;

but at times they may change the entire bulk of urine into a gelatinous mass. This condition likewise is essentially observed in cases of chyluria, but may possibly also occur in association with nephritis. Lostorfer[1] has reported an instance of this kind, in which fibrinous coagulation took place in the *clear* urine, which contained much albumin, but no blood. Post-mortem chronic inflammatory changes and amyloidosis of the kidneys was found, while the urinary passages proper were intact.

Nucleo-albumin.—The question whether or not nucleo-albumin is a normal constituent of the urine is still under dispute. Personal investigations have led me to the conclusion that with complicated methods and large amounts of urine—from 5 to 25 liters—it is always possible to demonstrate its presence both under physiological and pathological conditions. With the usual tests and smaller amounts of urine, however, negative results only are obtained in strictly normal individuals. According to my experience, trichloracetic acid, with which Stewart[2] claims to have obtained positive results in every one of the one hundred and fifty normal urines which he examined, does not precipitate nucleo-albumin when this is present in normal amounts. *A nucleo-albuminuria recognizable by the available tests does not exist under normal conditions.* Even under pathological conditions nucleo-albumin is by no means always found. Sarzin[3] thus was unable to demonstrate its presence in two hundred cases which he examined in Senator's clinic. Citron[4] arrived at similar results, and of several thousand urines which I have examined in this direction positive results were obtained in only a small percentage of cases. It is essentially met with in diseases which directly or indirectly involve the integrity of the epithelial lining of the uriniferous tubules or of the bladder. It has thus been frequently found in cases of acute nephritis and associated with febrile albuminuria, although its presence even then is not constant. In chronic nephritis it is more frequently absent than present. In cases of renal hyperæmia and cystitis the results are variable. In thirty-two icteric urines Obermayer[5] obtained positive results without exception, and it appears that in leukæmia nucleo-albumin is also quite constantly present. During the administration of pyrogallol, naphtol, corrosive sublimate, tar preparations, arsenic, etc., as well as in cases of poisoning with anilin and illuminating-gas, large amounts of the substance may be found.

According to my experience, nucleo-albumin is frequently obtained in cases of so-called functional albuminuria, and it is not uncommon to find that this is still present when serum-albumin and serum-globulin can no longer be demonstrated, even with the

[1] R. Lostorfer, Wien. klin. Woch., 1903, No. 7.
[2] D. D. Stewart, Med. News, 1894.
[3] D. Sarzin, Ueber Nucleo-albuminausscheidung, Diss., Berlin, 1894.
[4] Ueber Mucin im Harn, Diss., Berlin, 1886.
[5] Obermayer, Centralbl. f. klin. Med., 1892, vol. xiii. p. 1.

trichloracetic acid test. Nucleo-albuminuria may thus exist independently of the presence of the more common forms of albumin. This observation has also been made by Strauss, who found nucleo-albumin only in several cases of cystitis, in one case of chronic interstitial nephritis, and in one case of emphysema pulmonum with renal hyperæmia.

The existence of a hæmatogenic form of nucleo-albuminuria has thus far not been satisfactorily demonstrated. It has been assumed that its presence indicates increased epithelial desquamation in some portion of the urinary tract—in other words, that it is of cellular origin. Matsumoto, however, has shown that even though a urine containing numerous epithelial casts, renal epithelial cells, and leucocytes be allowed to stand for some time, a substance which can be precipitated with acetic acid either does not occur at all or only in very small quantity. He has rendered it very probable that the substance which can be precipitated from pathological urines by means of acetic acid is largely fibrinogen and euglobulin. He adds that nucleo-albumin may be present simultaneously, but in comparison to the other two substances it is of secondary importance and is rarely seen.

Histon and Nucleohiston.—Kolisch and Burian[1] were able to demonstrate the presence of histon in a case of leukæmia in which it was constantly present. More recently Krehl and Matthes[2] claim to have isolated the same substance in various febrile diseases, such as acute peritonitis, following appendicitis, in croupous pneumonia, erysipelas, and scarlatina. It is an albuminous body, and was first discovered by Kossel in the red blood-corpuscles of the goose. It exists in the leucocytes of human blood in combination with the acid leukonuclein, constituting the so-called nucleohiston of Lilienfeld.

It is not clear in what manner the histonuria is produced; so much, however, seems certain, that it is not solely dependent upon increased destruction of leucocytes.

Nucleohiston itself has been found in the urine in a case of pseudoleukæmia, by Jolles.[3]

Tests for Albumin.—The recognition of the various albuminous bodies which may occur in the urine is based partly upon their direct precipitation and partly upon color-reactions when treated with certain reagents.

The number of tests which have from time to time been suggested is large; many of them after a brief period of use have been discarded as useless or uncertain, while others have been employed only occasionally, and have not received the recognition which they

[1] R. Kolisch u. R. Burian, "Ueber d. Eiweisskörper d. leukämischen Harns," etc., Zeit. f. klin. Med., vol. xxix. p. 374.

[2] L. Krehl u. M. Matthes, "Ueber febrile Albumosurie," Deutsch. Arch. f. klin. Med., vol. liv. p. 508.

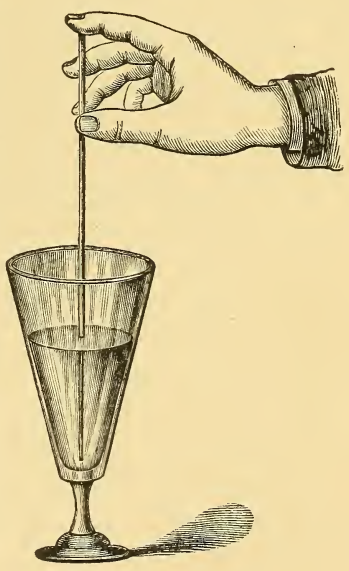
[3] A. Jolles, Ber. d. deutsch. chem. Gesellsch., vol. xxx. p. 172; Zeit. f. klin. Med., vol. xxxiv. p. 53.

deserve, from the fact that simpler tests exist, that they do not possess sufficient delicacy, or that in some instances it is too great. In the following pages no attempt is made to describe all of these tests, and attention will be directed only to those which are generally used, and which clinical experience has proved to be of value, precedence being given to those which have been longest in use. While some of these are applicable for demonstrating the presence of more than one form of albumin, special tests will also be described whereby the various albumins may be individually recognized.

In every case the urine should be carefully filtered, so as to free it from any morphological elements, etc., present. To this end, it is generally sufficient to pass the urine through one or two layers of Swedish filter-paper. Frequently, however, a clear specimen cannot be obtained in this manner; it is then advisable to shake the urine with burnt magnesia or talcum, or to mix it with scraps of filter-paper, when it is filtered as usual.

Tests for Serum-albumin.—THE NITRIC ACID TEST[1] (Fig. 111). —The value of this test, properly applied, cannot be overestimated, as it is not only simple, but yields an amount of information that can otherwise be gained only with difficulty. Usually the student is advised to make use of a test-tube partially filled with urine, along the sides of which concentrated, chemically pure nitric acid is allowed to flow, so as to form a layer at the bottom of the tube, when in the presence of serum-albumin a distinct white ring appears at the zone of contact between the two liquids (Heller's test). The pictures thus obtained cannot be compared, however, with those seen when the apparently trivial change is made of using a conical glass of about 2 ounces capacity instead of the test-tube. About 20 c.c. of urine are placed in the glass, when 6 to 10 c.c. of nitric acid are added by means of a pipette, which is carried to the bottom of the vessel; the acid is slowly allowed to escape by diminishing the pressure of the finger upon the tube. When this is carefully done the nitric acid forms a distinct zone beneath the urine. In the presence of albumin the white

FIG. 111.



Nitric acid test.

[1] J. F. Heller, Arch. f. physiol. u. path. Chem. u. Micros., 1852, vol. v. p. 169. A. Robin, Urologie clinique de la fièvre typhoide, Paris, 1877.

ring then appears, and varies in extent and intensity with the amount of albumin present (Plate XVIII., Fig. 1). If now the contents of the glass are allowed to stand undisturbed—and if small amounts are present, these appear only on standing for several minutes—it will be observed that the cloudiness gradually extends upward; and if much albumin is present, it may be seen to rise into the supernatant liquid in the form of small, irregular columns. This appearance is possibly referable to the partial decomposition of uric acid by means of nitric acid, nitrogen and carbon dioxide being set free, which, rising to the surface in the form of small bubbles, carry the nitric acid upward; coming into contact with albumin in solution, this is then precipitated.

An excess of uric acid is indicated by the appearance, within five to ten minutes after addition of the nitric acid, of a distinct ring in the clear urine, about 1 to 2 cm. above the zone of contact, which is similar in appearance to that due to albumin. If this ring (Plate XVIII., Figs. 1, 2, and 3), which has been appropriately compared to a communion wafer, does not appear within five to ten minutes, it may be assumed that uric acid is present in diminished amount. The degree of increase, on the other hand, may be determined by the size of the ring, it being presupposed that the same quantities of urine and of the reagent are employed in every case.

Should more than 25 grammes of urea be contained in a liter of the urine examined, an appearance like hoarfrost will be noted on the sides of the vessel, which is due to the formation of urea nitrate. Spangles of the same substance appear only in the presence of at least 45 grammes; and if 50 grammes or more of urea are contained in the liter, a dense mass of urea nitrate may be seen to separate out.

Biliary urine, when treated with nitric acid containing a little nitrous acid, shows the color-play referable to the action of nitric acid upon bilirubin (Plate XVIII., Fig. 4). The production of the colors (red, yellow, green, blue, and violet) takes place from above downward, the green color being the most characteristic; in the absence of the latter the presence of biliary pigment may be positively excluded. The presence of albumin is not objectionable, as the color-play takes place beneath the albuminous disk.

In normal urine a transparent ring is also obtained, presenting a peach-blossom-red; the intensity of this may vary, however, from a faint rose to a pronounced brick color, and is referable to normal urinary pigment (Plate XVIII., Fig. 5). In the presence of urobilin, on the other hand, this ring presents a distinct mahogany color.

Indican is indicated by the appearance of a violet ring (Plate XVIII., Fig. 2) situated above that referable to the normal urinary pigment. Its intensity varies with the amount present, from a light blue to a deep indigo.

The milky cloud at the zone of contact of the two fluids may be referable not only to the presence of serum-albumin, but also of

PLATE XVIII

FIG. 2.

FIG. 4.

FIG. 1.

FIG. 3.

FIG. 5.

globulin and albumoses (propeptones), while a negative reaction will generally indicate the absence of these bodies. That the uric acid ring will be mistaken for albumin is hardly likely if it is remembered that this never first appears at the zone of contact of the two fluids, but always in the uppermost portion of the urine. It is true that urines are occasionally observed in which the separation of uric acid takes place so suddenly that within a minute or two the entire urinous portion of the mixture is completely clouded, resembling the appearance presented by a highly albuminous urine. Such an excessive elimination of uric acid is uncommon, however, and it is to be remembered that with uric acid the cloudiness extends from above downward, and never from below upward, as is the case with albumin. Should any doubt be felt, it is only necessary to remove a few cubic centimeters of this cloudy urine by means of a pipette and to heat it gently in a test-tube, when the urine will clear up entirely if the precipitate is due to uric acid, while if caused by albumin it will remain or become more intense. Should the precipitate caused by nitric acid consist of albumoses, it will also clear up entirely, to reappear on cooling, the fluid at the same time assuming a markedly yellow color. The occurrence of a distinctly yellow color in the urine, moreover, which is only partially cleared upon the application of heat (and be it remembered that a much higher temperature is necessary for the solution of a precipitate referable to albumoses than of one due to urates), will indicate the existence of a mixed albuminuria—*i. e.*, the presence of coagulable albumin and albumoses.

Nitric acid may also cause a precipitation of certain resinous bodies, such as those contained in turpentine, balsam of copaiba and tolu, etc. If any doubt is felt, the mixture should be shaken with alcohol, when the precipitate caused by these substances is at once dissolved. The mucinous body—nucleo-albumin—which is at times found in

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DESCRIPTION OF PLATE XVIII.

The Nitric Acid Test as Applied to the Urine.

Fig. 1.—The light, colorless ring in the clear urine above shows a slight increase in the amount of uric acid; the large white band denotes a large amount of albumin, bordering upon a colored ring, referable partly to indican (blue) and partly to urorosein.

Fig. 2.—The light ring in the clear urine above denotes a slight increase in the amount of uric acid. The bluish-black band is referable to an enormous increase in the amount of indican. (Ileus.)

Fig. 3.—The broad, light band in the clear urine above is referable to an enormous increase in the amount of uric acid. (Laparotomy.)

Fig. 4.—The color-play referable to the presence of bilirubin is shown in a diagrammatic manner.

Fig. 5.—The colored ring is referable to the presence of normal urinary coloring-matter.

the urine is also precipitated by nitric acid, but need not occupy our attention at this place. From what has been said, it is manifest that the employment of the nitric acid test in the manner indicated furnishes much valuable information, and the adoption of the method as described not only by hospital students, but by general practitioners as well, cannot be too strongly urged.

Boiling Test.—A few cubic centimeters of urine are boiled in a test-tube and then treated with a few drops of concentrated nitric acid, no matter whether a precipitate has occurred upon boiling or not. If albumin is present, this will separate out as a flaky precipitate, which consists of serum-albumin frequently mixed with serum-globulin. It is true that albuminous urines will generally yield a precipitate on boiling alone; but it must be remembered that unless the reaction is decidedly acid a precipitation of normal calcium phosphate may occur, owing to the fact that the reaction of the urine upon boiling becomes less acid from escape of the carbonic acid held in solution. In urines presenting an alkaline or amphoteric reaction this is very frequently noted, and might give rise to confusion, as the precipitate due to calcium phosphate closely resembles that referable to albumin. Care must hence be taken to insure a distinctly acid reaction, which is best accomplished by the addition of nitric acid, when a precipitate referable to phosphates is at once dissolved, while one due to albumin remains, and may even become more marked. The quantity to be added should usually be equivalent to about 0.05 to 0.1 of the volume of urine. Under no condition should the acid be added before boiling, nor should the urine be boiled after its addition, as small amounts of albumin will otherwise be overlooked, owing to the fact that hot nitric acid dissolves the precipitate to a certain degree. If, after addition of the nitric acid the urine turns a distinct yellow, and if then upon cooling a white precipitate appears, the presence of albumoses may be inferred. Uric acid will probably never cause confusion, as this separates out only upon cooling, and then presents a dark-brown color. As in the case of the nitric acid test, so also here, a precipitation of certain resins is noted at times which may be recognized by their solubility in alcohol. Albumoses are also precipitated upon the application of heat, but such precipitates again dissolve when the temperature approaches the boiling-point (see page 502).

Should acetic acid be used instead of nitric acid, great care must be taken to avoid an excess, as otherwise the albumin will be dissolved. As this danger diminishes the greater the quantity of salts contained in the urine, it is advisable to treat the urine first with a few drops of acetic acid until a distinctly acid reaction is obtained, and then to add one-sixth its volume of a saturated solution of sodium chloride, magnesium sulphate, or sodium sulphate, when upon boiling a precipitation of the albumin will occur. Carried

out in this manner, the test is absolutely certain and will demonstrate even minimal amounts of albumin. If an equal volume of a saturated solution of common salt is added to the acidified urine, albumoses are also precipitated, but the precipitate dissolves on boiling.

The Potassium Ferrocyanide Test.—A few cubic centimeters of urine are *strongly* acidified with acetic acid (sp. gr. 1.064) and treated with a few drops of a 10 per cent. solution of potassium ferrocyanide, when, in the presence of but little albumin, a faint turbidity, or, if much albumin is present, a flaky precipitate, is noted, which is best recognized by comparison with a tube containing some of the pure filtered urine, both tubes being held against a black background. Concentrated urines should be previously diluted with water, as albumoses, like serum-albumin and serum-globulin, which may be precipitated in this manner, otherwise remain in solution. Here, also, as in the tests described, the presence of albumoses may be inferred if the precipitate disappears upon boiling, while a partial clearing up, on the other hand, indicates the presence of albumoses and coagulable albumin.

At times the addition of acetic acid by itself is followed by the appearance of a cloud in the urine, which may be due to urates or to urinary mucin (nucleo-albumin), as already mentioned. In such cases the urine should be refiltered, diluted with water, and the test again applied.

v. Jaksch advises the careful addition, by means of a pipette, of a few cubic centimeters of fairly concentrated acetic acid, to which a little potassium ferrocyanide has been added, when the albumin, as in Heller's test, is seen to form a ring at the zone of contact between the two fluids. Instead of potassium ferrocyanide, potassium platinocyanide may also be employed, and has the advantage that the test-solution is colorless.

The Trichloracetic Acid Test.[1]—This test is undoubtedly the most delicate of those so far described, but not so delicate that a trace of albumin or nucleo-albumin can be demonstrated in every urine. An experience based upon the examination of several thousand urines with this reagent warrants my speaking with a certain degree of confidence upon the subject. Very frequently it is possible with this method to demonstrate albumin in urines in which the more common tests yield negative results, but in which tube-casts may nevertheless be found upon microscopical examination. The test is applied as follows: by means of a pipette 1 or 2 c.c. of an aqueous solution of the reagent (sp. gr. 1.147) are carried to the bottom of a test-tube containing the carefully filtered urine, so as to form a layer beneath the urine. In the presence of albumin a white

[1] F. Obermayer, Wien. med. Jahrbüch, 1888, p. 375. D. M. Reese, Johns Hopkins Hosp. Bull., 1890.

ring will be seen to form at the zone of contact between the two fluids, varying in intensity with the amount of albumin present. So far as the test for albumin is concerned, this reagent possesses an advantage over nitric acid in that the colored rings, which are so confusing to the inexperienced, are commonly not observed. Serum-albumin, serum-globulin, and albumoses are precipitated, the presence of the latter being recognized, as in the previous tests, by the fact that the precipitate disappears upon boiling and reappears on cooling. A cloud, referable to uric acid, also appears if this is present in excessive amounts, but disappears upon the application of gentle heat. A previous dilution of the urine, moreover, guards against its occurrence.

Other tests have also been suggested for the detection of albumin in the urine, such as the metaphosphoric acid test, the phenol, tannic acid, and picric acid tests, that with Tanret's reagent, phosphotungstic and phosphomolybdic acids, and quite recently Spiegler's reagent.

Of these, only the picric acid and Spiegler's test will be considered.

Picric Acid Test.—The picric acid test is not applicable as a test for albumin as such, and is mentioned in this connection only because the same reagent is employed with Esbach's quantitative method. This is composed of 10 grammes of picric acid and 20 grammes of crystallized citric acid, dissolved in a liter of distilled water. If to this solution albuminous urine is added, the mixture is rendered turbid, and after some time a sediment which consists not only of albumins, but also of uric acid, kreatinin, and other extractives, will form at the bottom of the tube (see Quantitative Estimation of Albumin).

Spiegler's Test.[1]—Spiegler's reagent consists of 8 parts by weight of mercuric chloride, 4 parts of tartaric acid, and 200 parts of water, in which 20 parts of cane-sugar are further dissolved, so as to increase the specific gravity of the reagent and permit of its being employed, like Heller's test, even in concentrated urines. One-third of a test-tube is filled with the reagent, and the urine carefully placed above this by allowing it to flow slowly down the side of the tube; in the presence of albumin a sharply defined white ring will be observed where the two liquids are in contact. Peptone gives no reaction, while albumoses are precipitated and may be recognized as indicated above. Unfortunately the reagent will also precipitate nucleo-albumin.

Special Test for Serum-albumin.—Should it be desired, for any reason, to demonstrate serum-albumin alone, the urine is rendered amphoteric or faintly alkaline with sodium hydrate, and is then saturated with magnesium sulphate in substance, in order to remove any globulin. The filtrate is strongly acidified with acetic acid,

[1] Spiegler, Wien. klin. Woch., 1892, vol. v. p. 26.

when a flaky precipitate, appearing upon boiling, will indicate the presence of serum-albumin.

Patein's albumin differs from the common serum-albumin in being soluble in acetic acid.[1]

Very often, as in the examination for sugar, it is necessary to remove any coagulable albumin that may be present, to which end the urine is rendered distinctly acid with acetic acid and boiled. An examination of the filtrate with potassium ferrocyanide, if the amount of acetic acid added was just sufficient, will then yield a negative result (see page 497).

Quantitative Estimation of Albumin.—For the quantitative estimation of albumin a large number of methods have been devised, which fact in itself is sufficient to indicate that the majority of them, at least, are unsatisfactory.

Old Method by Boiling.—If comparative results only are desired, a definite amount of urine is boiled after acidifying with acetic acid; the albumin is allowed to settle for twenty-four hours. For this purpose Neubauer suggests the use of glass tubes measuring one-half to three-quarters of an inch in diameter, which are closed at the lower end with a cork. Ordinary test-tubes answer perfectly well, but care should be taken that the same quantity of urine is used in each case. The tubes are corked and kept for several days for comparison. The results, of course, express only the relative amount of albumin present, and it should be remembered that the error incurred may amount to as much as 30 or even 50 per cent. of the quantity that is found by gravimetric analysis. This is owing to the fact that sometimes the albumin separates out in large flakes, and at other times in small flakes, and that the degree of precipitation is also influenced by the specific gravity of the supernatant urine.

Volumetric Method of Wassiliew.[2]—This method can be recommended for the quantitative estimation of albumin, as it is both simple and accurate.

Ten to 20 c.c. of urine, which are best diluted to 50 c.c. with distilled water, are treated with 2 drops of a 1 per cent. aqueous solution of true yellow (Echtgelb[3] of Grübler), and then titrated with a 12.5 per cent. solution of salicyl-sulphonic acid until a distinct brick-red color is obtained. The number of cubic centimeters of the reagent employed, multiplied by 0.00006, will indicate the amount of albumin in the 10 or 20 c.c. of urine examined. If the urine is alkaline, it should first be slightly acidified with acetic acid.

[1] Patein, "Aceto-soluble Albumin in the Urine," Compt. rend. de l'Acad. des Sci., 1889. Coplin, Phila. Med. Jour., 1899, p. 957.

[2] Wassiliew, Eshenedelnik, 1896, No. 26; St. Petersburg. med. Woch., 1897, Beilage, p. 4.

[3] Echtgelb is a mixture of amidoazobenzol disulphonate and sodium monosulphonate.

Esbach's Method.[1]—The reagent is composed of 10 grammes of picric acid and 20 grammes of citric acid, dissolved in 1000 c.c. of distilled water. Special tubes, termed albuminimeters (Fig. 112), are employed, which bear two marks, one, *U*, indicating the point to which urine must be added, and one, *R*, the point to which the reagent is added. The lower portion of the tube up to *U* bears a scale reading from 1 to 7, corresponding to the amount of albumin pro mille. The tube is filled to *U* with the filtered albuminous urine, and the reagent added until the point *R* is reached. The tube is then closed with a stopper, inverted twelve times, and set aside for twenty-four hours. At the expiration of this time serum-albumin, serum-globulin, and albumoses, as well as uric acid and kreatinin, will have settled, when the amount pro mille in grammes may be directly read off from the scale. A few precautions must, however, be observed in order to obtain as accurate results as possible. The reaction of the urine should be acid, and if this is not the case acetic acid is added. Its specific gravity should not exceed 1.006 or 1.008, the proper density being obtained by diluting with water. The amount of albumin in the specimen should not exceed 0.4 per cent.; if more be present, as determined by a preliminary test, the urine should be diluted. Most important, furthermore, is the temperature of the room. This should be 15° C.; variations from this point are apt to give rise to inaccurate results, which, according to Christensen, may amount to 100 per cent. in the case of a deviation of only 5 degrees C. It is thus clear that as generally employed in the clinical laboratory the method will only give approximate results.

Fig. 112.

Esbach's albuminimeter.

The Differential Density Method.[2]—More accurate results may be obtained with the following method, which is based upon the diminution in the specific gravity of the urine after the removal of all albumin, and its comparison with the specific gravity observed before. To this end, the urine is treated with a sufficient amount of acetic acid to insure complete precipitation of the albumin (see below), when its specific gravity is noted. It is then brought to the boiling-point, care being taken to guard against evaporation by placing the urine in an ordinary medicine-bottle; this is closed with a rubber stopper that has been previously boiled in a solution of sodium hydrate and washed free from alkali, the stopper being tightly fastened with a cord or wire. Thus prepared, the bottle is kept in boiling water for ten to fifteen minutes. The urine is filtered on cooling, evaporation being again carefully guarded against by filter-

[1] Guttmann, Berlin. klin. Woch., 1886, vol. xxiii. p. 117.
[2] Huppert u. Záhòr, Zeit. f. physiol. Chem., 1886, vol. xii. pp. 467 and 484.

ing into a bottle through a funnel which has been passed through a closely fitting stopper; the funnel is kept covered with a plate of glass. The specific gravity is then again determined, and it is best in both cases to use a pyknometer. (An accurate hydrometer, graduated to the fourth decimal, may, however, also be used.) The decrease in the specific gravity, multiplied by 400, will indicate the number of grammes of albumin in 100 c.c. of urine.

Gravimetric Method.—If accuracy is required, the amount of albumin must be determined gravimetrically as follows: a certain quantity of urine, after having been acidified with an amount of acetic acid sufficient to insure complete precipitation of all albumin, is boiled; the albumin is then filtered off, dried, and weighed. For this purpose, 500 to 1000 c.c. of carefully filtered urine should be available. A specimen of this, if already acid, is placed in a test-tube, in boiling water, until coagulation takes place, when it is further heated over the free flame and filtered. The filtrate is then tested with acetic acid and potassium ferrocyanide. Should no albumin be thus demonstrable, the entire amount of urine is treated in the same manner, and requires no further addition of acetic acid. If, however, the test yields a positive result, it is apparent that the urine was not sufficiently acid. The entire volume is then treated with a 30 to 50 per cent. solution of acetic acid, drop by drop, the mixture being thoroughly stirred and specimens tested from time to time, as described. When, finally, the urine remains clear or shows only a faint turbidity, 100 c.c. or less, according to the amount of albumin present, are first heated in boiling water until the albumin begins to separate out in flakes, and then carefully brought to the boiling-point over the free flame. The supernatant urine is decanted through a filter, which has been previously dried at 120° to 130° C. and accurately weighed, when the whole amount of the precipitate is brought upon the filter. Any albumin remaining in the beaker is detached from its sides by means of a glass rod tipped with a piece of rubber tubing, and collected by the aid of hot water. The entire precipitate is now thoroughly washed with hot water until the washings no longer become turbid when treated with a drop of nitric acid and silver nitrate; in other words, until the chlorides have been completely removed. The precipitate is further washed with alcohol and finally with ether to remove any fats that may be present, when it is dried at 120° to 130° C. until a constant weight is reached. If still greater accuracy is required, the dried and weighed precipitate is incinerated to determine the amount of mineral ash in combination with the albumin, which is then deducted from the total weight. The most accurate results are obtained if not more than 0.2 to 0.3 gramme of albumin is contained in the amount of urine employed. A smaller quantity than 100 c.c. should hence be used if a previous test with Esbach's albuminimeter shows a higher percentage.

A glass-wool filter insures a more rapid process of drying—twenty-four to thirty hours; but care must then be had that this is properly prepared, so as to guard against a loss of the wool while washing.

Method by Centrifugation.—This presupposes a constant speed, and hence an electrical centrifuge is a prerequisite, which is an objection to the general adoption of the method. But even with its aid results are not always obtained which are accurate.

Test for Serum-globulin and its Quantitative Estimation.—To test for serum-globulin the urine is rendered alkaline by the addition of ammonium hydrate, any phosphates that may thus be thrown down being filtered off on standing. The urine is then treated with an equal volume of a saturated solution of ammonium sulphate, when the occurrence of a precipitate will indicate the presence of the globulin. Ammonium urate may likewise separate out, but this occurs later.

According to Paton, the following test may also be employed: the urine after having been rendered alkaline with sodium hydrate,—any phosphates which may separate out are filtered off,—is carefully poured down the side of a test-tube containing a saturated solution of sodium sulphate, so as to form a layer above this, when in the presence of serum-globulin a white ring will appear at the zone of contact.

If a *quantitative estimation* of the globulin is to be made, the precipitate thus obtained, after about one hour's standing, is collected on a dried and weighed filter, and washed thoroughly with a one-half saturated solution of ammonium sulphate until a specimen of the washings treated with acetic acid and potassium ferrocyanide no longer gives a precipitate. It is then treated as directed in the method employed for the quantitative estimation of serum-albumin.

Tests for Albumoses.—A small amount of urine is strongly acidified with acetic acid and treated with an equal volume of a saturated solution of common salt. In the presence of albumoses a precipitate occurs, which dissolves on boiling and reappears on cooling. If serum-albumin also be present, which is usually the case, the hot liquid must be filtered. The albumoses are found in the filtrate and appear on cooling. If the *hot* filtrate, moreover, is rendered alkaline with a solution of sodium hydrate, a red color develops upon the addition of a very dilute solution of cupric sulphate, added drop by drop (biuret reaction). On boiling with *Millon's reagent* a red color is also obtained. This reagent is prepared by dissolving 1 part of mercury in 2 parts of nitric acid of a specific gravity of 1.42, and diluting with 2 volumes of distilled water.

Salkowski's Method.—Fifty c.c. of urine are acidified in a

[1] E. Salkowski, "Ueber d. Nachweis d. Peptons (Albumosen) im Harn u. d. Darstellung d. Urobilins," Berlin. klin. Woch., 1887, p. 353.

beaker with 5 c.c. of hydrochloric acid, and precipitated with phosphotungstic acid, the mixture being heated over the free flame, when in a few minutes the precipitate will form a resinous mass which closely adheres to the bottom of the vessel. The supernatant fluid is decanted, and the mass at the bottom, which now becomes granular, washed twice with distilled water, which is likewise removed by decantation. The precipitate is then covered with about 8 c.c. of distilled water, and treated with 0.5 c.c. of a sodium hydrate solution (sp. gr. 1.16). Upon shaking the beaker the mass will dissolve, the solution assuming a dark-blue color. This is heated on the free flame until the blue color turns to a dirty, grayish-yellow; the solution at the same time becomes turbid, but at times may turn yellow and remain clear. This discoloration may be hastened by the further addition of a few drops of sodium hydrate solution. As soon as this point has been reached, some of the liquid is placed in a test-tube, allowed to cool, and then treated with a very dilute solution of cupric sulphate (1 to 2 per cent.) drop by drop; in the presence of peptones the solution assumes a bright-red color, which may be brought out still more strongly if the specimen is now filtered. If albumin or much mucin is present, these bodies must first be removed (see pages 499 and 506); but the quantity of urine employed is so small that the mucin can usually be disregarded. With this method, which occupies only about five minutes, 0.015 gramme of peptones pro 100 c.c. may be demonstrated without difficulty.

Salkowski has recently pointed out that urines which are very rich in urobilin, as in pneumonia, may give rise to the biuret reaction even when albumoses are absent. The coloring-matter, it is true, may be removed entirely by precipitation with lead acetate or subacetate, but unfortunately a portion of the albumoses is also carried down, and the substance may thus escape detection when present only in small amounts. He hence suggests that smaller quantities of urine, such as 10 c.c., be employed in the test. The reaction is then not so well marked, but the results are more reliable.

Bang's Method.—This method has recently been introduced, and is said to be free from the objections attaching to the one proposed by Salkowski. Ten c.c. of urine are heated in a test-tube with 8 grammes of finely powdered ammonium sulphate until the salt has been dissolved; the fluid is then boiled for a moment. The hot fluid is centrifugated for one-half to one minute, the supernatant fluid poured off, and the sediment stirred with alcohol in an agate mortar. The alcohol is poured off, and the residue dissolved in a little water; the solution is boiled and filtered, and the filtrate tested with sodium hydrate solution and cupric sulphate as described. Should the urine be especially rich in urobilin—*i. e.*, manifesting a well-marked fluorescence with zinc chloride and ammonia—it is

best to extract the final aqueous solution with chloroform by shaking, and to pour off the supernatant fluid, when this is tested with cupric sulphate. In this manner it is possible to demonstrate the presence of albumoses in a dilution of 1 : 4000–5000. Other constituents of the urine, with the exception of hæmatoporphyrin, do not interfere with the test. Should hæmatoporphyrin be present, however, which may be suspected if a red alcoholic extract is obtained, the urine must first be precipitated with barium chloride. The filtrate, which contains the albumoses, is then examined as described.

If a centrifuge is not available, the urine is boiled with the ammonium sulphate, when a portion of the albumoses will remain on the sides of the tube as a sticky mass. This is washed with alcohol, and if necessary with chloroform, dissolved in water, and tested for biuret.

The alcoholic extract may also be used for testing for urobilin. To this end, it is only necessary to add a few drops of a solution of zinc chloride, when in the presence of urobilin a beautiful fluorescence will be observed. The test is extremely delicate.[1]

Examination for True Peptone.[2]—To demonstrate the presence of true peptone in the urine, about 300 c.c. of filtered acid urine are saturated on a water-bath with ammonium sulphate at a temperature between 60° and 70° C. On cooling, the mixture is filtered, the filtrate is alkalinized with a dilute solution of sodium carbonate, again saturated between 60° and 70° C. with ammonium sulphate, filtered on cooling, the filtrate neutralized with very dilute acetic acid, again saturated with the salt between 40° and 50° C., and finally again filtered on cooling. The final filtrate is diluted with an equal volume of distilled water and treated with a freshly prepared solution of tannic acid, which is added drop by drop, care being taken to avoid an excess. The precipitate is filtered off the next day, dried in the desiccator upon the filter, powdered, and covered in a porcelain crucible with a small amount of baryta-water to which a little finely powdered baryta is added. The mixture is placed on a boiling water-bath for three minutes, and after one or two hours it is filtered. If necessary, the solution is decolorized with neutral lead acetate. The biuret test is finally applied, and if positive, indicates the presence of peptone in the sense of Kühne.

Tests for Bence Jones' Albumin.—The presence of Bence Jones' albumin is usually discovered on slowly heating the urine to the boiling-point. It will then be noted that at a temperature of from 50° to 60° C. a more or less intense, milky turbidity develops, which on subsequent boiling either disappears entirely or partially, and reappears on cooling. The degree to which the urine clears on

[1] E. Bang, "Eine neue Methode zum Nachweis d. Albumosen im Harn," Deutsch. med. Woch., 1898, p. 17.

[2] Ito, loc. cit.

boiling differs in different cases. As I have just stated, the turbidity may disappear entirely; but, on the other hand, urines are met with in which even a partial clearing can scarcely be made out. This is apparently dependent upon the degree of acidity of the urine, the amount of mineral salts and of urea present, and probably also upon other and still unknown factors.

Upon the addition of a drop of nitric acid to a few cubic centimeters of such urine a temporary turbidity develops, which disappears on shaking, but persists if a little more of the acid is added. If now the mixture is heated, the albumin first coagulates to a dense mass; on boiling, this dissolves, and after a while the liquid becomes almost entirely clear, while the turbidity returns, as before, on subsequent cooling. Similar reactions are obtained with all the common reagents for albumin.

For its complete identification, the albumin should be isolated and further examined as follows: larger amounts of urine are precipitated by the addition of one and one-half to two volumes of 96 per cent. alcohol, or by treating with two volumes of a saturated solution of ammonium sulphate. In either event the total amount of albumin is thrown down. This is then washed with alcohol and ether, and dried over sulphuric acid. To purify the substance, it is dissolved in boiling water, by the aid of a few drops of a dilute solution of sodium carbonate, and dialyzed to running and then to distilled water until free from mineral salts. It is then reprecipitated with alcohol (if necessary, after the addition of a drop or two of a dilute solution of hydrochloric acid), washed with absolute alcohol and ether, and dried. Thus purified, the albumin is practically insoluble in distilled water or saline solution at ordinary temperature, and only sparingly so at the boiling-point. In boiling water, however, it dissolves with comparative ease after the addition of a few drops of sodium carbonate solution. On neutralization no precipitate occurs if a sufficient amount of water is present. If such a neutral solution is heated, no change occurs; but if it is now acidified and a certain amount of salt added, the typical reaction appears on heating, viz., precipitation between 50° and 60° C. (even between 40° and 50° C. if a sufficient amount of salt is present), clearing on boiling, and reprecipitation on cooling.

On digestion with pepsin-hydrochloric acid, as I have said, a proto-albumose is obtained among the early products of digestion, while a hetero-albumose is not formed.

Test for (Mucin) Nucleo-albumin.—It has been generally supposed that the substance which is precipitated on adding strong acetic acid to certain pathological urines, when diluted two or three times with water, is nucleo-albumin, the precipitate being soluble or largely so in an excess of the reagent. Matsumoto,[1] however, has recently

[1] Matsumoto, "Ueber d. durch Essigsäure ausfällbare Eiweissubstanz in pathologischen Harnen," Deutsch. Arch., 1902, vol. xxv. p. 398.

pointed out that the substance which is precipitated in this manner is largely a mixture of fibrinogen (fibrinoglobulin) and euglobulin. Nucleo-albumin may be present at the same time, but it is rare, and its quantity in comparison to the two albumins mentioned insignificant.

To demonstrate the presence of nucleo-albumin, it is necessary to salt out the albumins with ammonium sulphate (half saturation is sufficient), and then to ascertain whether any precipitation occurs within the limits of precipitation of nucleo-albumins. Matsumoto gives these as 0.1 to 0.8 (lower limit) and 1.6 and 2.2 (upper limit). Its limits of precipitation are the lowest of the known albumins.[1]

Whether or not *Ott's test*[2] in the light of this work can still be relied upon as a test for the demonstration of nucleo-albumin may be questioned. It is conducted as follows: A few cubic centimeters of urine are treated with an equal volume of a saturated solution of common salt, when Almén's solution, which consists of 5 grammes of tannic acid, 10 c.c. of a 25 per cent. solution of acetic acid, and 240 c.c. of 40 to 50 per cent. alcohol, is slowly added. The development of a precipitate was regarded as evidence of the presence of nucleo-albumin.

In order to remove nucleo-albumin from the urine, this is treated with neutral lead acetate, an excess of the reagent being carefully avoided. If it is desired to test for peptones, the filtrate is then treated with hydrochloric acid and the process continued as described above.

Test for Hæmoglobin.—The diagnosis of hæmoglobinuria is based upon the demonstration of hæmoglobin, viz., methæmoglobin, in the urine in solution, in the absence of red corpuscles, or at least in the presence of only a very small number, so that an examination in the latter direction is also an important factor.

Bloody urine is generally turbid, and may vary in color from bright red to almost black.

Oxyhæmoglobin, as such, can only be recognized by the spectroscope; it gives rise to the appearance of two bands of absorption, situated between D and E, as described in the chapter on the Blood.

The urine to be examined spectroscopically should be rendered feebly acid by means of acetic acid, and placed before the open slit of the spectroscope in a test-tube, beaker, or similar vessel, when the two bands of oxyhæmoglobin will be seen, either at once or upon carefully diluting with distilled water. If ammonium sulphide is now added, the spectrum of reduced hæmoglobin will be obtained. It must be remembered, however, that more commonly the spectrum of methæmoglobin is seen in cases of hæmoglobinuria.

[1] Limit of precipitation of fibrinogen, 1.5 : 1.7–2.5 : 2.7; of fibrinoglobulin, 2.2 : 2.9; of euglobulin, 2.8 : 3.3; of pseudoglobulin, 3.4 : 4.6.

[2] A. Ott, Centralbl. f. inn. Med., 1895, vol. xvi. p. 38.

The following tests, which will also indicate the presence of blood coloring-matter, cannot be employed to decide the nature of the pigment present, as methæmoglobin and oxyhæmoglobin will both react in the same manner.

Heller's Test.[1]—A small amount of the urine, or still better a portion of the sediment, is made strongly alkaline with sodium hydrate and boiled. On standing, a deposit of basic phosphates forms, which in the presence of blood coloring-matter presents a bright-red color. This is referable to the formation of hæmochromogen, as may be shown by spectroscopic examination. Thus controlled, the test is extremely sensitive, and still yields a positive result when the chemical test alone leaves one in doubt.[2] The deciding band is the first between D and E. Care should be had, however, that the solution is cold, as otherwise the hæmochromogen is transformed into hæmatin in alkaline solution. At times, when the urine contains a large amount of coloring-matter (bile-pigment, etc.), it may be difficult to determine the exact color of the sediment. In such cases the subsequent examination with the spectroscope,—the lensless instrument of Hering or that of Browning suffices,—is invaluable. In the absence of such apparatus the procedure of v. Jaksch may be employed. To this end, the phosphatic deposit is filtered off and dissolved in acetic acid, when if blood-pigment is present the solution becomes red, the color gradually vanishing upon exposure to the air. The delicacy of the test is such that oxyhæmoglobin can still be demonstrated in a dilution of 1 : 4000.

The Guaiacum Test.[3]—A mixture of equal parts of tincture of guaiacum and oil of turpentine (which has been ozonized by exposure to the air) is allowed to flow slowly along the side of a test-tube upon the urine to be examined, in such a manner as to form a distinct layer above the urine. In the presence of blood-pigment a white ring, which gradually turns blue, will be seen to form at the zone of contact.

Donogany's Test.[4]—About 10 c.c. of urine are treated with 1 c.c. of a solution of ammonium sulphide and the same amount of pyridin, when in the presence of blood a more or less intense orange color develops, especially if looked at from above, against a white background. In doubtful cases the examination is to be controlled by a spectroscopic examination of the resulting mixture. If blood-pigment is present, the spectrum of hæmochromogen is obtained. Should the ammonium sulphide and pyridin be old, a green or brown color is imparted to the urine, which changes to yellow upon the addition of ammonium hydrate.

[1] J. F. Heller, Zeit. d. K. K. Gesellsch. d. Aerzte zu Wien, 1858, No. 48.
[2] V. Arnold, Berlin. klin. Woch., 1898, p. 283.
[3] Almén, see Hammarsten, Lehrbuch der physiol. Chem., 3d ed. p. 488.
[4] Z. Donogany, "Darstellung d. Hæmochromogen als Reaction auf Blut," etc., Virchow's Archiv, vol. cxlviii. p. 234.

Test for Fibrin.—Fibrin usually occurs in the urine in the form of distinct clots, the nature of which may be determined by thoroughly washing with water, when they are dissolved by boiling in a 1 per cent. solution of soda or a 5 per cent. solution of hydrochloric acid. On cooling, this solution is tested as for serum-albumin.

Test for Histon.—The urine of twenty-four hours is first examined for albumin, and this removed if present. It is then precipitated with 94 per cent. alcohol, the precipitate washed with hot alcohol and dissolved in boiling water. Upon cooling, the solution thus obtained is acidified with hydrochloric acid and allowed to stand for several hours. During this time a cloudiness, referable to a large extent to uric acid, develops, which is filtered off, and the filtrate is precipitated with ammonia. The precipitate is collected on a small filter and washed with ammoniacal water until the washings no longer give the biuret reaction. It is then dissolved in dilute acetic acid and the solution tested with the biuret test; if this yields a positive result, and if coagulation occurs upon the application of heat, the coagulum being soluble in mineral acids, the presence of histon may be inferred.

CARBOHYDRATES.

The carbohydrates which may occur in the urine are glucose, lactose, maltose, dextrin, levulose, certain pentoses, and animal gum.

Glucose.—Through the researches of Wedenski, v. Udranszky, and others,[1] we know that traces of glucose may be encountered in the urine under strictly normal conditions. The amount, however, is extremely small, and special methods are necessary in order to demonstrate its presence. With the usual clinical tests normal urine is apparently free from sugar unless unduly large amounts have recently been ingested. In that event a certain amount of glucose is eliminated in the urine, constituting the so-called *digestive glucosuria* of Claude Bernard.[2]

The normal limit to the assimilation of glucose on the part of the body economy is subject to considerable variation. Some observers thus report that the ingestion of such large amounts as 200 and 250 grammes does not lead to glucosuria, while others have found sugar in the urine after the administration of 100 grammes. In view of the possible relation existing between diabetes and a lowered limit to the assimilation of glucose in apparently normal individuals, or at least in persons in whose urine glucose cannot be constantly demonstrated, this question has created much interest within the last few years and has called forth a large amount of work. The major-

[1] A. Baumann, Ber. d. Deutsch. chem. Ges., 1886, vol. xix. p. 3218. N. Wedenski, Zeit. f. physiol. Chem., 1889, vol. xiii. p. 122. K. Baisch, Ibid., 1894, vol. xviii. p. 193, and 1895, vol. xix. p. 348.

[2] Claude Bernard, Compt. rend. de l'Acad. des Sci., 1859, vol. xlviii. p. 673.

ity of investigators are now in accord in regarding as abnormal a glucosuria that follows the ingestion of 100 grammes of chemically pure glucose.

The method usually employed in order to ascertain the power of assimilation for glucose on the part of an individual is the following:

The patient receives 100 grammes of glucose, in substance, dissolved in 500 c.c. of water, on an empty stomach, and is instructed to pass his water hourly during the following four to five hours. During this time, moreover, no food is to be taken. The individual specimens, as well as the urine which has been passed during the night, are then tested with Trommer's and Nylander's tests, with the fermentation test, and with phenyl-hydrazin. A positive result, however, is recorded only when sugar can be demonstrated with the fermentation test.

Cane-sugar and larger amounts of glucose have also been used; but it is better, on the whole, as Strauss has pointed out, to give glucose, and not to exceed the dose of 100 grammes.

Especially interesting are the results which have been obtained in various diseases of the liver, to which organ the important function of preventing an undue accumulation of sugar in the blood has been repeatedly ascribed. Bierens de Haën [1] thus reports that of twenty-nine cases of various hepatic diseases he found sugar in eighteen after the administration of 150 grammes of cane-sugar; and v. Jaksch [2] claims to have obtained positive results in fifteen cases of phosphorus poisoning out of forty-three. Strauss,[3] on the other hand, states that he found sugar in only two of his thirty-eight cases, and has collected one hundred and seven additional cases from the literature, in only fourteen of which could sugar be demonstrated. If we add these together, we have one hundred and forty-five cases of various hepatic diseases, with negative results in 88.9 per cent. Referring to the contradictory results obtained, Strauss points out that these may have been accidental in part, but that the interpretation which has been offered by v. Jaksch and de Haën may not have been correct. It is thus possible that in his cases of phosphorus poisoning other factors besides the changes in the liver, such as the action of the poison upon the nervous system, etc., played a *rôle*, as a digestive glucosuria may also occur in connection with other forms of intoxication, as in fevers, following the administration of large doses of diuretin, in acute alcoholism, etc., in which the liver is not the only organ that is involved. Strauss further shows that great care must be exercised in the selection of the material for such investigations, and believes that errors referable to this source may have been incurred by Bierens de Haën. He thus

[1] J. C. Bierens de Haën, "Ueber alimentäre Glycosurie bei Leberkranken," Arch. f. Verdauungskrank., vol. iv. p. 4.

[2] v. Jaksch, "Alimentäre Glycosurie," Prag. med. Woch., 1895, Nos. 27, 31, and 32.

[3] H. Strauss, "Leber und Glycosurie," Berlin. klin. Woch., 1898, p. 1122.

cites two cases of hypertrophic cirrhosis, associated with delirium tremens, in which small amounts of sugar could be demonstrated in the urine a few days after recovery from the delirium, while shortly after negative results only could be obtained. The lowering effect of alcoholism upon the limit to the assimilation of glucose is a well-known phenomenon, and it would be erroneous to conclude that because alcoholism may call forth organic changes in the liver the digestive glucosuria in such cases is referable to such alterations. Without entering further into the question at this place, it appears that diseases of the liver *per se* do not materially lessen the assimilation of glucose, and that other forces are at the disposal of the body to supply the glycogen-forming or retaining power of the liver when this becomes insufficient, and that these also must be at fault when a digestive glucosuria is observed in association with hepatic disorders.

The association of digestive glucosuria with various diseases of the nervous system has been carefully studied by v. Jaksch,[1] Strümpell, H. Strauss,[2] von Oordt, Geelvink, and Arndt.[3] From the work of these investigators it appears that digestive glucosuria is rarely seen in spinal diseases, and is decidedly more common in functional diseases of the central nervous system than in organic affections. Of thirty cases of tabes examined by Strauss, digestive glucosuria resulted in only one after the administration of 100 grammes of glucose, and in that one case a family history of diabetes existed. In sixteen further cases examined by J. Strauss negative results were obtained. In the neuroses a positive result was noted in forty-two out of two hundred and ten cases which I have been able to collect from the literature. Most frequently it is met with in the traumatic neuroses, in which Strauss observed the phenomenon in 37.5 per cent. of his forty cases; while in the non-traumatic forms only 14.4 per cent. were insufficient in this respect. Of the organic diseases of the central nervous system, it appears that diffuse cerebral lesions referable to alcohol and syphilis are more likely to give rise to this form of glucosuria than the more localized lesions. In general paresis digestive glucosuria is thus not uncommon (H. Strauss, Arndt), but it is only possible to draw definite deductions from the study of a large amount of clinical material. Small series like that of J. Strauss do not give a proper idea of actual conditions, as he, for example, obtained negative results in all of 10 cases.

In his examination of 5 cases of idiocy and 23 cases of imbecility, J. Strauss obtained positive results in only 2 of the imbeciles after the administration of 100 grammes of glucose; in both of the posi-

[1] v. Jaksch, loc. cit.

[2] H. Strauss, "Zur Lehre v. d. neurogenen u. d. thyreogenen Glycosurie," Deutsch. med. Woch., 1897, pp. 275 and 309.

[3] M. Arndt, "Ueber alimentäre Glycosurie bei Neuropsychosen," Berlin. klin. Woch., 1898, p. 1085.

tive cases the glucosuria was transitory and associated with the existence of nervous excitability. Bergenthal observed alimentary glucosuria in 6 cases out of 20.

In Basedow's disease digestive glucosuria has also been noted in a large number of cases by Chvostek, Kraus and Ludwig, Strauss, Goldschmidt and Stern. Especially interesting in this connection is the fact that digestive glucosuria may be induced by the administration of thyroid extract, viz., thyroidin or iodothyrin in apparently normal persons. Bettmann[1] thus noted glucosuria after the ingestion of 100 grammes of glucose in 12 of 25 healthy individuals who had been treated for a week with the products in question.

A digestive glucosuria is further observed in numerous febrile diseases, such as pneumonia, typhoid fever, acute articular rheumatism, scarlatina, tonsillitis, etc. The amount of sugar usually found varies from 0.5 to 3 per cent.; larger amounts may, however, also be encountered, and one case is on record in which 8 per cent. was present.[2]

Very common also, as I have indicated, is the digestive glucosuria of drinkers, and there can be little doubt that the habitual ingestion of large quantities of beer and spirits will in the course of time lead to a more than temporary enfeeblement of the carbohydrate metabolism. In the course of his investigations in this direction, Krehl[3] found that among the Jena students the proportion of those in whose urine sugar appeared apparently varied with different kinds of beer, but was much greater after morning drinking. Of fourteen who drank bock or export beer in the morning, five had glucosuria. After the evening drinking, amounting in one case to 7 liters, of nineteen only one had sugar in the urine, and with Bavarian beer one of eleven.

Of diseases of the skin, digestive glucosuria is notably associated with psoriasis; and it is interesting to note that the same disease is not infrequently seen in diabetic patients. Gross thus records five cases, in four of which the psoriasis had existed for many years before the appearance of diabetic symptoms. Similar instances are recorded by Strauss, Grube, Polotebuoff, Nielssen, Schütz, and others. Nagelschmidt[4] was able to produce glucosuria by the ingestion of 100 grammes of glucose in eight cases out of twenty-five.

During pregnancy digestive glucosuria is also frequently observed, and is by some regarded as a fairly constant symptom and of diagnostic importance. The amount is variable, and while Lanz[5] records

[1] Bettmann, "Ueber d. Einfluss d. Schilddüsenbehandl. auf d. Kohlendydratstoffwechsel," Berlin. klin. Woch., 1897, p. 518.

[2] R. v. Bleiweis, "Ueber alimentäre Glycosurie e saccharo bei acuten, fieberhaften Infektionskrankheiten," Centralbl. f. inn. Med., 1900, No. 2.

[3] Krehl, "Alimentäre Glycosurie nach Biergenuss," Centralbl. f. inn. Med., 1897, No. 40.

[4] Nagelschmidt, "Psoriasis und Glycosurie," Berlin. klin. Woch., 1900, No. 2.

[5] Lanz, Wien. med. Presse, 1895, vol. xxxvi.

one case in which 29.6 grammes of glucose were found after the ingestion of 100 grammes, such figures are certainly uncommon, and as a general rule less than 3 grammes are recovered from the urine. After delivery the power of assimilation for glucose no longer appears to be subnormal.

A digestive glucosuria has further been observed in acute and chronic lead poisoning, poisoning with nitrobenzol, anilin dyes, opium, atropin, and carbon monoxide; in the early stages (the first twelve days) of acute phosphorus poisoning; in the febrile form of *embarras gastrique*, etc. In these conditions, however, the phenomenon has received little attention.

In patients afflicted with disease of the heart, liver, and kidneys Gobbi[1] observed a digestive glucosuria, after the ingestion of from 100 to 200 grammes of glucose, if diuretin was at the same time administered.

Very important is the fact that in diabetes mellitus the sugar may at times disappear from the urine, while its elimination is replaced by an excessive excretion of uric acid or phosphates. In such cases glucosuria may be produced with ease by the ingestion of 100 grammes of glucose, a point which may be of value in diagnosis. The exhibition of such amounts of sugar in true diabetes while glucosuria already exists will cause an increased elimination, while this apparently does not occur in other forms of glucosuria. Interesting further is the fact that in diabetic patients an increased elimination of sugar can be produced by the administration of full doses of copaiba. That this drug is in itself capable of lowering the limit to the assimilation of glucose has recently been shown by Bettmann. A digestive glucosuria was thus produced in four patients out of twelve to whom copaiba had been given for one week in amounts varying from 1 to 2 grammes.

The digestive glucosuria to which reference has been made in the preceding pages is generally spoken of as the *digestive glucosuria e saccharo*. Similar results have been obtained after the administration of starches in excess, viz., 150–200 grammes. But while a digestive glucosuria e saccharo is regarded only as a possible indication of a pathological alteration of the carbohydrate metabolism, it is generally thought that every *glucosuria ex amylo*[1] is indicative of a definite disturbance in the sense of diabetes, unless special factors, such as an increase of the surrounding temperature, diminished radiation of heat, or complete lack of muscular activity, are active. Strauss, however, has shown that in cases in which a somewhat more than temporary predisposition toward glucosuria e saccharo exists, as in alcoholics, for example, a coincident tendency toward glucosuria ex amylo may likewise be demonstrated. As a

[1] G. Gobbi, "La glucosuria da diuretina," Il Policlinico, 1900, No. 5.
[2] E. Külz, Beiträge zur Pathol. u. Therap. d. Diabetes, Marburg, 1874, vol. i. p. 110.

result of his experiments he concludes that the difference between the digestive glucosuria e saccharo and glucosuria ex amylo is essentially a question of degree. *Cœteris paribus*, it appears that harmful influences of a slight character lead to glucosuria e saccharo, while grave insults call forth glucosuria ex amylo. It results practically that the prognosis in those cases in which digestive glucosuria follows a temporary insult is far better than when the carbohydrate metabolism is permanently damaged, and especially when a glucosuria ex amylo accompanies a glucosuria e saccharo. In the first instance it is scarcely likely that true diabetes will develop in the course of time, while in the latter this is at least possible.

Aside from the digestive form of glucosuria which has just been considered, and which is produced artificially, an idiopathic transitory form is also known to occur. A *transitory glucosuria*, apparently of central origin, is thus noted in connection with lesions affecting the central as well as the peripheral nervous system, such as tumors and hemorrhages at the base of the brain, lesions of the floor of the fourth ventricle, cerebral and spinal meningitis, concussion of the brain, fracture of the cervical vertebræ, tetanus, sciatica; following epileptic, hystero-epileptic, and apoplectic seizures, mental shock produced by railroad accidents (traumatic neuroses), etc.; mental strain and worry, fatigue, and anxiety. Glucosuria following epileptic and apoplectic attacks, however, does not appear to be so common as is generally believed. v. Jaksch was unable to demonstrate the presence of sugar in fifty recent cases of hemiplegia, and in a large number of cases of epilepsy, with urines voided within the first few hours following the seizure I have reached only negative results.

In Basedow's disease transitory glucosuria may also occur, and it is well established that a relation may exist between the disease in question and the complex of symptoms is designated as diabetes mellitus.[1]

Siegmund noted a transitory glucosuria in 52.38 per cent. of general paretics, in 7.4 per cent. of epileptics, and in 3.77 per cent. of dementia cases, while it was not observed in other mental diseases. In reference to the post-epileptic glucosuria which has been noted by some of the older observers more especially, an analysis of their work has led me to the conclusion that their inferences were scarcely justifiable, as a wholly satisfactory proof of the presence of sugar has not been furnished.[2]

In cases of cholelithiasis, contrary to what has been maintained by one or two observers, glucosuria is unusual.

[1] Dumontpallier, "Goiter exophthalmique et glycosurie," Compt. rend. d. l. soc. d. Biol., 1867. O'Neill, "Exophthalmic Goitre and Diabetes occurring in the same Person," Lancet, 1878, Pt. 1. p. 9. S. Bettmann, Münch. med. Woch., 1896, vol. xliii. Nos. 49 and 50. E. Grawitz, Fortsch. d. Med., 1897, vol. xv, K. Osterwald, Inaug. Diss., Göttingen, 1898. H. Stern, Jour. Am. Med. Assoc., 1902, vol. xxxix. p. 972.

[2] See, also, Araki, Zeit. f. phys. Chem., vol. xv. p. 363.

It is well known that Claude Bernard experimentally produced a transitory glucosuria by puncturing a certain spot in the floor of the fourth ventricle, the supposed origin of the hepatic vasomotor nerves, and it is not improbable that this neurotic form of glucosuria is due to some direct or reflex influence affecting that portion of the medulla.

The transitory glucosuria occasionally observed in acute febrile diseases, such as typhoid fever, scarlatina, measles, cholera, diphtheria, influenza, and especially malaria, particularly during convalescence, may possibly be referable to the action of ptomaïns or leukomaïns upon this centre. Seegen reports five cases of malaria with "diabetes" in which *both conditions* disappeared under the administration of quinin. In diphtheria glucosuria appears to be of common occurrence. Binet thus obtained a positive result in twenty-nine cases out of seventy; twenty-seven times in severe infections out of thirty-eight, and twice in mild cases out of thirty-two. I have personally found a transitory glucosuria in four cases out of thirty-two; the infection in these was of moderate severity. Hibbard and Morrissey arrived at similar results.[1]

A glucosuria of toxic origin has been noted in cases of poisoning with curare, chloral hydrate, sulphuric acid, arsenic, alcohol, carbon monoxide, morphin, etc., and even after simple transfusion of normal salt solution into the blood. Phloridzin, a glucoside obtained from the bark of the root of the apple tree, will likewise cause sugar to appear in the urine. The glucosuria thus produced is, however, only temporary, and ceases upon withdrawal of the drug.[2] Of interest is the glucosuria which occasionally follows the administration of thyroid extract or of iodothyrin, as there is evidence to show that in such cases a special predisposition to glucosuria exists. When carried to an extreme degree true diabetes may develop, which subsequently cannot be arrested by withdrawal of the substance.[3]

The occurrence of a transitory glucosuria under the conditions above mentioned, and which may be met with in almost any disease, moreover, while interesting from a theoretical standpoint, must in the majority of instances be regarded as a medical curiosity only, and it is but rarely possible to draw either diagnostic, prognostic, or therapeutic conclusions from its existence.

A *persistent form of glucosuria* is noted in connection with certain lesions of the brain, especially those affecting the floor of the fourth ventricle, and is at times of considerable value in diagnosis. This is also observed after removal of the thyroid gland, and in cases in which thyroid extract has been administered in unduly large amount.

[1] C. M. Hibbard and M. J. Morrissey, "Glycosuria in Diphtheria," Jour. Exper. Med., vol. iv. p. 137.

[2] Zuntz, "Zur Kenntniss d. Phloridzindiabetes," Du Bois' Archiv, 1895, p. 570.

[3] H. Strauss, "Neurogene and thyreogene Glucosurie," Deutsch. med. Woch., 1897, Nos. 19 and 20.

A continuous elimination of sugar, however, is noted principally in the complex of symptoms to which the term *diabetes mellitus* has been applied.

Diabetes mellitus is essentially a persistent form of glucosuria associated with the occurrence of a more or less intense polyuria and a greatly increased elimination of all the metabolic products normally found in the urine, with the exception of uric acid, which is usually present in diminished amount. In the more advanced cases acetonuria, lipuria, and lipaciduria may also exist. Diabetes, however, is not a persistent form of glucosuria in an absolute sense of the word, as periods may occur in the course of the disease when glucose is temporarily absent.

The quantity of sugar excreted may be very large, and 180 to 360 grammes pro die are amounts which may be frequently observed. This quantity may diminish to zero under various conditions, such as the occurrence of intercurrent diseases, but often also without any apparent cause, and not infrequently in the condition which has been termed diabetic coma. Cases are also observed in which from beginning to end mere traces are eliminated, the total amount of sugar not exceeding a few grammes, while the course of the disease rapidly tends toward a fatal termination, *so that the severity of the pathological process cannot be measured by the amount of sugar eliminated.* A few years ago I had occasion to observe a diabetic patient in whom for months a daily examination of the urine never revealed the presence of more than 5 to 10 grammes of sugar, and in whom death occurred after eighteen months.

At the same time it should be remembered that diabetes cannot be excluded by one or even more negative urinary examinations, and the value of repeating such examinations three or four hours after the exhibition of 100 grammes of glucose, as indicated, cannot be too strongly urged.

Clinicians are in the habit of determining the severity of a case, to a certain extent at least, from the condition of the urine under a diet free from starches and sugars, and generally regard those cases as the more serious in which the glucosuria does not disappear under a diet of this character, while a more favorable prognosis is given if the sugar disappears. It should be remembered, however, that there are numerous exceptions to this rule, and that a light case,—*i. e.*, one in which the sugar disappears under appropriate dietetic treatment,—may suddenly exhibit symptoms seen only in the most severe forms, or succumb to one of the numerous intercurrent maladies, while apparently severe cases may assume the more benign type.

It may not be out of place in this connection to say a few words regarding the specific gravity of the urine. While usually very high, varying between 1.030 and 1.060, as pointed out in the chapter

on Specific Gravity, comparatively low figures are noted at times, such as 1.012, corresponding to a quantity of urine not exceeding 1000 c.c., and implying, of course, a diminished elimination of solids. This is especially marked in those cases described by Hirschfeld,[1] in which, as pointed out in the chapter on Urea, the resorption of nitrogenous material from the digestive tract is below the normal. Polyuria, a fairly constant symptom of the more common types of diabetes mellitus, is much less pronounced in Hirschfeld's form, and may be altogether absent, although it is true that this may occur in ordinary diabetes also.

The simultaneous occurrence of glucosuria, acetonuria, lipuria, and lipaciduria (which see) is probably always indicative of true diabetes.

It is, of course, impossible to enter here into a detailed consideration of the origin of diabetes. Suffice it to say that a persistent glucosuria, aside from nervous influences, may be referable, on the one hand, to an inability on the part of the liver to transform into glycogen all of the sugar which is carried to this organ ; or, on the other hand, to an inability on the part of the muscular system of the body to utilize all the sugar sent to it. Accordingly, we may distinguish between a *hepatogenic* and a *myogenic diabetes*. As a matter of fact, cases are seen, usually belonging to the milder form of the disease, in which the sugar may be temporarily caused to disappear from the urine by muscular exercise. On the other hand, again, cases are seen, and unfortunately only too frequently, in which, notwithstanding a total abstinence from carbohydrates and a free indulgence in muscular exercise, the sugar does not disappear from the urine. In such cases it is permissible to speak of a hepatogenic combined with a myogenic diabetes.

Within recent years it has been shown that pancreatic disease is frequently associated with diabetes, and while the number of cases in which no pancreatic lesions are discovered is still too large to warrant the conclusion that disease of this organ is invariably associated with glucosuria, it still must be admitted that lesions of the pancreas are the more frequently met with in diabetes the more carefully the organ is examined. So much appears to be certain, that diabetes *may* be produced by pancreatic disease. As to the manner, however, in which such a result can occur we are in ignorance. In this connection it is interesting to note that, according to Opie, disease of the areas of Langerhans more especially is associated with the clinical picture of diabetes, while lesions affecting the secreting portion of the gland only do not influence the carbohydrate metabolism.[2] These observations of Opie have been largely confirmed by other observers.

[1] F. Hirschfeld, "Ueber eine neue klinische Form d. Diabetes," Zeit. f. klin. Med., vol. xix. pp. 294 and 325.

[2] Opie, Jour. Exper. Med., 1901, vol. v. p. 527.

Hirschfeld pointed out the fact that while in the majority of diabetic patients the proteid food ingested is quite satisfactorily utilized, the assimilation of fats and albumins is much below normal in others, and particularly so in cases of diabetes associated with pancreatic disease (see also Urea). Observations in this direction are as yet very scanty, so that a definite opinion cannot be expressed regarding the utility in diagnosis of investigations similar to those of Hirschfeld. I have had occasion to observe a diabetic patient for some time in whom, notwithstanding that conclusions were reached similar to those of Hirschfeld, the existence of pancreatic disease could not be determined post mortem.

Whether or not a renal and a thyroigenic diabetes also exists, as has recently been suggested, remains an open question.[1] That Basedow's disease may be associated with diabetes mellitus I have already pointed out.

Tests for Sugar.—The tests for sugar usually employed in the clinical laboratory depend upon the following properties of sugar:

1. In the presence of alkalies it acts as a reducing agent upon certain metallic oxides, such as those of copper and bismuth (Fehling's, Trommer's, Böttger's, and Nylander's tests).

2. In the presence of yeast (Saccharomyces cerevisiæ) it undergoes fermentation, with the formation of alcohol, carbonic acid, succinic acid, glycerin, and a number of other bodies, such as amyl alcohol, etc. (fermentation test).

3. With phenylhydrazin sugar forms an insoluble crystalline compound—phenylglucosazon.

4. Solutions of glucose turn the plane of polarized light to the right, from which property glucose has also received the name *dextrose*.

In every case the urine should first be tested for the presence of albumin, which should be removed by boiling.

Trommer's Test.[2]—A few cubic centimeters of urine are strongly alkalinized with sodium hydrate solution, and treated with a 5 per cent. solution of cupric sulphate, added drop by drop, until the cupric oxide formed is no longer dissolved. The mixture is carefully heated, when in the presence of sugar a yellow precipitate of cuprous hydroxide is formed, which gradually settles to the bottom as a sediment of red cuprous oxide.

It is important to note that while sugar, unless present in mere traces, can readily be detected in this manner, other substances are

[1] Diabetes: J. Seegen, Die Zuckerbildung im Thierkörper, Berlin, 1890, p. 260. v. Noorden, Pathol. d. Stoffwechsels, Berlin, 1893. Seegen, "Ueber d. Zuckergehalt d. Blutes von Diabetikern." Wien. med. Woch., 1886, Nos. 47 and 48. F. W. Pavy, "Ueber die Behandlung von Diabetes mellitus," Verhandl. d. X. Internat. Med. Congr., 1891, II., Abt. 5, p. 80. P. F. Richter, "Nierendiabetes," Deutsch. med. Woch., 1899, p. 840.

[2] C. Trommer, Annal. d. Chem. u. Pharm., 1841, vol. xxxix. p. 361.

or may be present in the urine, such as uric acid, kreatin and kreatinin, allantoin, nucleo-albumin, milk-sugar, pyrocatechin, hydrochinon, and bile-pigment, which likewise reduce cupric oxide. Following the ingestion of benzoic acid, salicylic acid, glycerin, chloral, sulphonal, etc., reducing substances also appear. These may generally be disregarded, it is true, if care is taken *not to boil* the urine after the addition of the cupric sulphate, as the precipitation of cuprous oxide in the presence of sugar takes place before this point is reached. Unfortunately, however, the test when thus applied yields negative results, or results which are doubtful, if traces only are present, so that it cannot be utilized, as a rule, in the study of transitory or digestive glucosuria.

Fehling's Test.[1]—This is a modification of the test just described, and can be recommended only with the same restrictions.

Two solutions are employed, which must be kept in separate bottles, the one containing 34.64 grammes of crystallized cupric sulphate, dissolved in 500 c.c. of distilled water, and the other 173 grammes of potassium and sodium tartrate and 125 grammes of potassium hydrate, dissolved in an equal volume of water. Equal parts of the two solutions, mixed in a test-tube and diluted with four times as much water, are boiled, when a small amount of urine is added. In the presence of sugar a precipitate of the yellow hydroxide of copper or of red cuprous oxide will be produced; but *care should be taken only to warm, and not to boil the solution after addition of the urine.*

Not infrequently it will be observed that upon standing, when no precipitation has occurred previously, the blue color of the mixture changes to an emerald green, while the solution at the same time becomes turbid. Such a phenomenon should not be referred to the presence of sugar, as it is in all probability due to the action of other reducing substances, such as those mentioned above.

Böttger's Test with Nylander's Modification.[2]—A few cubic centimeters of urine are treated with *Almén's solution* in the proportion of 11 : 1. This is prepared by dissolving 4 grammes of potassium and sodium tartrate, 2 grammes of bismuth subnitrate, and 10 grammes of sodium hydrate in 90 c.c. of water, heating the solution to the boiling-point and filtering upon cooling, when it should be kept in a colored glass bottle. The mixture of urine and Almén's fluid is thoroughly boiled, when in the presence of sugar a grayish, dark-brown, and finally a black precipitate, consisting of bismuthous oxide or of metallic bismuth, is obtained. Albumin, if present, must first be removed, as, owing to the sulphur contained in the albuminous molecule, alkaline sulphides would be formed upon boiling, and, acting upon the bismuth, give rise to the

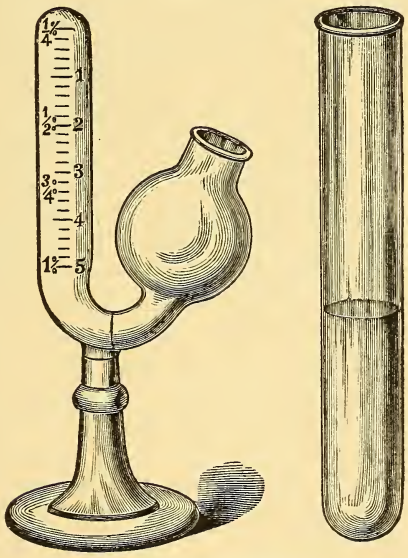
[1] H. Fehling, Annal. d. Chem. u. Pharm., 1849, vol. lxxii. p. 106.
[2] E. Nylander, Zeit. f. physiol. Chem., 1883, vol. viii. p. 175.

formation of black bismuth sulphide, which might be mistaken for metallic bismuth. Rhubarb-pigment, as well as melanin and melanogen (which see), and free hydrogen sulphide must also be absent, as misleading results will otherwise be obtained.

Nylander's test, as well as those of Trommer and Fehling, is, however, not without objections, as a partial reduction of the bismuth subnitrate may be produced by other substances, such as kairin, tincture of eucalyptus, turpentine, and large doses of quinin.

Fermentation Test.[1]—A small piece of ordinary compressed yeast is shaken with some of the suspected urine and a test-tube filled with the mixture, to which some mercury is added. The tube is then inverted into a vessel containing mercury, and allowed to stand in a warm place (22°–28° C.). If sugar is present, fermentation will occur in the course of twelve hours, and the carbon dioxide formed rise to the top of the tube, gradually displacing more and more of the urine or mercury as the amount of the gas increases. It is easy to demonstrate that the gas thus formed is carbon dioxide by introducing a small piece of caustic soda into the urine, when, owing to absorption of the carbon dioxide, the liquid will again rise in the tube. Very convenient for this purpose also are the saccha-

Fig. 113.



Einhorn's saccharimeter.

rimetric tubes of Einhorn (Fig. 113) or Lohnstein[2] (Fig. 115), which are employed as just described, a little mercury being poured

[1] M. Einhorn, Virchow's Archiv, 1885, vol. cii. p. 263.
[2] Lohnstein, Berlin. klin. Woch., 1898, p. 866.

into the bent limb to guard against escape of gas. As the yeast itself, however, may give rise to the formation of a little gas in the absence of sugar, it will always be well to make a control-test with normal urine—*i. e.*, to prepare a similar tube with normal urine mixed with yeast, and to allow this to stand at the same temperature. If a positive result is thus obtained, there can be no doubt as to the presence of a fermentable substance in the urine. This, however, is not necessarily glucose, as other carbohydrates, such as lactose, maltose, and levulose, may likewise undergo fermentation. Still, if large amounts of gas are obtained, and if Trommer's test also yields a positive result, it will be fairly safe to regard the substance present as glucose.

Phenylhydrazin Test.[1]—As originally proposed by v. Jaksch, the test is conducted as follows: 6 to 8 c.c. of urine are treated with 0.4 to 0.5 gramme of phenylhydrazin hydrochlorate and 1 gramme of sodium acetate, and warmed until the salts have been dissolved, a little water being added if necessary. The tube is placed in boiling water for twenty to thirty minutes, and then transferred to a beaker filled with cold water. If sugar is present in moderate amounts, a bright-yellow crystalline deposit will at once be thrown down and partly adhere to the sides of the tube. But even in the presence of mere traces a careful microscopical examination will reveal the presence of crystals of phenylglucosazon (Plate XIX.). These are seen singly or arranged in bundles and sheaves composed of delicate bright-yellow needles which are insoluble in water.

Still more convenient is the following modification of the test, as suggested by Cipollina:[2] 5 drops of pure phenylhydrazin, 0.5 c.c. of glacial acetic acid or 1 c.c. of 50 per cent. acetic acid are placed in a test-tube together with 4 c.c. of urine. The mixture is boiled for about one minute over a small flame, while shaking so as to avoid bumping as much as possible; 4 or 5 drops of sodium hydrate solution (specific gravity 1.16) are added, but the solution must remain acid; the boiling is continued for a few seconds and the mixture then allowed to cool. The rapidity with which the glucosazon crystals separate out depends somewhat upon the specific gravity of the urine. If this is low, they form in a few minutes, even though the amount of sugar does not exceed 0.05 per cent. If, on the other hand, the specific gravity is high, yellow balls and Stechapfelformen result, while typical rosettes develop only after twenty to thirty minutes, and at times one is even then left in doubt as to the result. If the urine contains more than 0.2 per cent. of sugar, even though the specific gravity be high, the formation of typical crystals occurs within a few minutes. If with this modification no crystals are

[1] v. Jaksch, Zeit. f. klin. Med., 1886, vol. xi. p. 20.
[2] A. Cipollina, Deutsch. med. Woch., 1901, vol. xxvii. p. 334.

PLATE XIX.

Phenyl-Glucosazon Crystals obtained from a Diabetic Urine.

obtained at the expiration of an hour, we may infer that no sugar is present.

This test, properly applied, is undoubtedly not only the most delicate, but at the same time the most reliable, as no other substances which may be present in the urine, excepting maltose and certain pentoses, will give rise to the formation of an osazon. Hence, whenever doubt is felt as to the nature of a substance reacting in a positive manner with the reagents described above, recourse should be had to this test. It has been stated that maltose forms an exception; this, however, will never become embarrassing, as the microscopical appearance of the maltosazon crystals differs from that of the phenylglucosazon. The melting-point of phenylglucosazon (205° C.), moreover, is about 15 degrees higher than that of the maltosazon—190°–191° C. To determine this point, it is necessary to filter off the osazon, and, after washing with water, to dissolve it upon a filter by means of a little hot alcohol. From this alcoholic solution it is reprecipitated by water, when it may be collected and dried over sulphuric acid. The melting-point is then determined according to the usual methods.

The pentosazons also can be readily distinguished from glucosazon by their melting-points (which see).

The amount of lactose which may be found in the urine is far too small to give rise to the formation of an osazon when the test is directly applied to the urine.

With the conjugate glucuronates phenylhydrazin also combines to form crystalline compounds, but these may likewise be distinguished by their melting-points and the form of the crystals. Such compounds, moreover, are usually not present in amounts sufficient to give rise to confusion (see Glucuronic Acid).

Polarimetric Test.—Glucose turns the plane of polarized light to the right, but the same may be said of maltose, the degree of polarization of which is even more marked, so that it may be impossible to state in a given case whether such rotation is referable to a large quantity of glucose or to a smaller quantity of maltose. The latter substance, however, occurs in the urine but rarely, and may be recognized not only by the microscopical appearance of its osazon, but also by the fact that its power of reduction is increased in the presence of sulphuric acid and by the application of heat.

An error which may further arise with the employment of the polarimetric method is referable to the fact that if glucose is present in only small amounts, while the urine contains large quantities of β-oxybutyric acid, the latter turning the plane of polarized light to the left, it may happen that the rotation in this direction will neutralize or even counterbalance any rotation to the right which may be due to glucose. In such cases, however, the urine will react in a

positive manner with the other reagents described, and the fermented urine will, moreover, turn the plane of polarization still more strongly to the left, indicating the presence of a dextrorotatory substance, and in all probability of glucose.

The delicacy of this method varies with the instrument employed; the figures given below were obtained with the apparatus of Lippich, which yields the best results.

(For a description of this method see the Quantitative Estimation of Sugar by Means of the Polarimeter.)

TABLE SHOWING THE DELICACY OF THE TESTS DESCRIBED.

Trommer's test	0.0025 per cent.
Fehling's test	0.0008 "
Nylander's test	0.025 "
Fermentation test	0.1–0.05 "
Phenylhydrazin test	0.025–0.05 "
Polarimetric test	0.025–0.05 "

TABLE SHOWING THE BEHAVIOR OF THE VARIOUS FORMS OF SUGAR WHICH MAY OCCUR IN THE URINE TOWARD THE TESTS DESCRIBED.

	Trommer's, viz., Fehling's test.	Nylander's test.	Fermentation test.	Phenylhydrazin test.	Polarimetric test.
Glucose.	Positive reaction.	Positive reaction.	Positive reaction.	Positive reaction; melting-point 205° C.	Rotation toward the right.
Levulose.	Positive reaction.	Positive reaction.	Positive reaction.	Same osazon obtained as with glucose, only more rapidly.	Rotation toward the left.
Maltose.	Positive reaction.	Positive reaction.	Positive reaction.	A maltosazon is formed; melting-point 190°–191° C.	Rotation toward the right.
Lactose.	Positive reaction.	Positive reaction.	No reaction or only a very faint one.	No reaction in the concentration in which it may occur in the urine; melting-point 200° C.	Rotation toward the right; increased by boiling with a 2.5 per cent. solution of sulphuric acid.
Laiose.	Positive reaction on boiling only; 1.2–1.8 per cent. more is obtained than by the polarimeter.	Positive reaction.	No reaction.	With phenylhydrazin a yellowish-brown, noncrystallizable oil is obtained.	No reaction, or rotation toward the left.

Clinically, it is unimportant to search for minute traces of sugar, such as may be found in every normal urine, and the reader is referred to special works on physiological chemistry for a consideration of the methods generally employed (see method of Baumann and v. Udranszky.

Quantitative Estimation of Sugar.—The methods used in the quantitative estimation of sugar are essentially based upon the qualitative tests described.

FEHLING'S METHOD.[1]—Fehling's solution prepared as described above is of such strength that the copper contained in 10 c.c. is completely reduced by 0.05 gramme of glucose. If then urine is carefully added to this quantity until complete reduction takes place, the amount of sugar contained in a given specimen of urine can be readily calculated according to the following equation :

$$y : 0.05 : : 100 : x ; \text{ and } x = \frac{5}{y},$$

in which y indicates the number of cubic centimeters of urine required to reduce the 10 c.c. of Fehling's solution, and x the amount of sugar contained in 100 c.c. of urine.

As the best results are obtained only if from 5 to 10 c.c. of urine are used in one titration, it is usually necessary to dilute the urine to the required degree ; in the determination of this point the specific gravity may serve as a guide. As a general rule, urines of a specific gravity of 1.030 should be diluted five times, and if the density is still higher ten times. To be certain that the proper degree of dilution has been reached, 5 c.c. of Fehling's solution are treated with 1 c.c. of the diluted urine, a little caustic soda solution and distilled water being added to make in all about 25 c.c. This mixture is thoroughly boiled ; if the fluid still remains blue, another 1 c.c. of diluted urine is added, and so on, until the last two tests differ by 1 c.c. of urine, the last cubic centimeter added causing a separation of cuprous oxide. In this manner the percentage of sugar may be approximately determined. Albumin, if present, must first be removed by boiling.

Ten c.c. of Fehling's solution diluted with 40 c.c. of water are placed in a porcelain dish and boiled. While boiling, the diluted urine is added from a burette, 0.5 c.c. at a time, when, as a rule, the precipitated cuprous oxide will rapidly settle, so that gradually a white bottom may be seen through the blue field, the color of which becomes less and less intense upon the further addition of urine until finally the solution is almost colorless. When this point is reached the urine is added drop by drop until the decolorization is complete. The degree of dilution multiplied by 5 and the result divided by the number of cubic centimeters of diluted urine employed will then indicate the percentage-amount of sugar. In the table on page 524 the percentage results corresponding to the number of cubic centimeters of undiluted urine employed will be found.

Unfortunately, it is difficult, as a general rule, to determine exactly the point when all the copper has been reduced—*i. e.*, the point at which the blue color has entirely disappeared. When it is thought that this has been reached, about 1 c.c. should be filtered through

[1] Loc. cit.

thick Swedish filter-paper, and the filtrate (which must be absolutely clear) acidified with acetic acid and treated with a drop or two of a solution of potassium ferrocyanide. If unreduced copper is still present in the solution, a brown color will result, indicating that sufficient urine has not been added. But if, on the other hand, no brown discoloration is noted, it is possible that the desired point has been passed, when the titration should be repeated. At times the precipitate will not settle at all, and even pass through the filter, so that it is practically impossible to determine the end of the reaction. In such cases the following procedure, suggested by Cause, will be found of value:

Ten c.c. of Fehling's solution are diluted with 20 c.c. of distilled water and treated with 4 c.c. of a 0.05 per cent. solution of potassium ferrocyanide. While boiling, the diluted urine is added drop by drop until the blue color entirely disappears. A precipitate does not form with this method.

SUGAR.—*Quantity of Glucose pro liter, corresponding to the number of cubic centimeters used for the complete reduction of 10 cubic centimeters of Fehling's solution.*

	1	$\frac{1}{10}$	$\frac{2}{10}$	$\frac{3}{10}$	$\frac{4}{10}$	$\frac{5}{10}$	$\frac{6}{10}$	$\frac{7}{10}$	$\frac{8}{10}$	$\frac{9}{10}$
1	50.00	45.44	41.68	38.46	35.70	33.32	31.24	29.40	27.76	26.30
2	25.00	23.80	22.72	21.72	20.84	20.00	19.22	18.50	17.84	17.24
3	16.66	16.00	15.62	15.14	14.15	14.28	13.88	13.50	13.14	12.82
4	12.50	12.18	11.90	11.62	11.36	11.10	10.86	10.62	10.40	10.20
5	10.00	9.80	9.60	9.42	9.24	9.08	8.92	8.76	8.62	8.50
6	8.32	8.18	8.06	7.92	7.80	7.68	7.56	7.44	7.34	7.24
7	7.14	7.04	6.94	6.86	6.78	6.66	6.56	6.48	6.40	6.32
8	6.24	6.16	6.08	6.02	5.94	5.88	5.80	5.74	5.68	5.60
9	5.54	5.48	5.42	5.36	5.30	5.24	5.20	5.16	5.12	5.06
10	5.00	4.94	4.90	4.82	4.78	4.76	4.70	4.66	4.62	4.58
11	4.54	4.50	4.46	4.42	4.38	4.34	4.30	4.26	4.22	4.20
12	4.16	4.14	4.12	4.08	4.04	4.00	3.98	3.96	3.92	3.86
13	3.84	3.80	3.78	3.76	3.74	3.70	3.68	3.66	3.62	3.58
14	3.56	3.54	3.52	3.48	3.46	3.44	3.42	3.40	3.36	3.34
15	3.32	3.32	3.28	3.26	3.24	3.22	3.20	3.18	3.16	3.14
16	3.12	3.10	3.08	3.04	3.04	3.02	3.00	2.98	2.96	2.94
17	2.94	2.92	2.90	2.88	2.86	2.84	2.82	2.82	2.80	2.78
18	2.76	2.76	2.74	2.72	2.70	2.70	2.68	2.64	2.64	2.64
19	2.62	2.62	2.60	2.60	2.58	2.56	2.56	2.54	2.52	2.52
20	2.50	2.50	2.48	2.48	2.44	2.42	2.42	2.40	2.40	2.38
21	2.38	2.36	2.34	2.34	2.32	2.32	2.30	2.30	2.28	2.28
22	2.26	2.26	2.24	2.24	2.22	2.22	2.20	2.20	2.18	2.18
23	2.16	2.16	2.14	2.14	2.12	2.12	2.12	2.10	2.10	2.10
24	2.08	2.08	2.06	2.06	2.06	2.04	2.04	2.02	2.02	2.02
25	2.00	1.98	1.98	1.96	1.96	1.96	1.94	1.94	1.92	1.92
26	1.92	1.92	1.90	1.90	1.88	1.88	1.88	1.86	1.86	1.86
27	1.84	1.82	1.82	1.82	1.82	1.80	1.80	1.80	1.80	1.80
28	1.78	1.76	1.74	1.74	1.74	1.74	1.74	1.74	1.74	1.72
29	1.72	1.70	1.70	1.70	1.70	1.68	1.68	1.68	1.68	1.66
30	1.66	1.66	1.65	1.63	1.63	1.62	1.62	1.62	1.62	1.62

In order to obtain reliable results, however, the Fehling solution must be prepared with great care and its strength determined. This may be done in the following manner: 0.2375 gramme of pure crystallized cane-sugar, dried at 100° C., is dissolved in 40 c.c. of distilled water, to which 22 drops of a 0.1 per cent. solution of sulphuric acid have been added. This solution is kept on the boiling

water-bath for an hour, when it is allowed to cool and diluted to 100 c.c. with distilled water. Twenty c.c. of this solution will then contain exactly 0.05 gramme of glucose, corresponding to 10 c.c. of Fehling's solution, if this is of the required strength. If too strong, so that 21 c.c. of the sugar solution, for example, are required to obtain a complete reduction of the copper, the strength of Fehling's solution may be determined according to the equation : $20 : 0.05 : : 21 : x$; and $x = 0.0525$. If too weak, on the other hand, so that 19 c.c., for example, are required, its strength is similarly determined : $20 : 0.05 : : 19 : x$; and $x = 0.0475$.

KNAPP'S METHOD.[1]—This method is said to be more satisfactory than that of Fehling. Daylight is not necessary; the method is simpler, and it is applicable even in cases in which the amount of sugar is small; and the solution keeps for a long while.

The principle of the method depends upon the observation that mercuric cyanide in alkaline solution is reduced to metallic mercury in the presence of sugar. The solution required should contain 10 grammes of chemically pure, dry mercuric cyanide and 100 c.c. of a solution of sodium hydrate (sp. gr. 1.145) to the liter. Twenty c.c. of this solution correspond to 0.05 gramme of glucose.

Method.—Twenty c.c. of the solution are placed in a small retort and diluted with 80 c.c. of water. If we have reason to suppose that the urine contains less than 0.5 per cent. of sugar, 40 to 60 c.c. are sufficient. The solution is then heated to the boiling-point, when the diluted urine (see below) is added, at first 2 c.c. at a time, then 1 c.c., 0.5 c.c., 0.2 c.c., and 0.1 c.c., as the final point is approached. After each addition the solution is boiled for one-half minute. As the end-reaction is approached the solution clears, and the mercury, together with the phosphates, settles to the bottom. The final point is determined by placing a drop of the supernatant fluid upon a piece of clean, white Swedish filter-paper, and holding this first over a bottle containing concentrated hydrochloric acid and then over one containing a saturated solution of hydrogen sulphide. If all the mercuric cyanide has not been reduced, a yellow spot will result, the color of which becomes the more manifest if it is compared with one which has not been exposed to the action of hydrogen sulphide. As soon as the mercury is entirely reduced the reading is taken.

Example.—Supposing that 15 c.c. of urine have been required, the corresponding amount of sugar is then found according to the following equation, 20 c.c. of Knapp's solution requiring 0.05 gramme of sugar for its reduction :

$$15 : 0.05 : : 100 : x; \quad 15x = 5; \quad \text{and } x = 0.333 \text{ per cent.}$$

[1] K. Knapp, Annal. d. Chem. u. Pharm., 1870, vol. cliv. p. 252.

Precautions : 1. Albumin must first be removed.

2. The urine should not contain more than 0.5 to 1 per cent. of sugar. The urine is hence diluted, if necessary, as with Fehling's method.

Differential Density Method.[1]—This method is very useful in clinical work, and should be preferred to the more uncertain titration with Fehling's solution, unless considerable experience has been acquired with the method.

The specific gravity of the urine is accurately ascertained by means of a pyknometer, or a hydrometer graduated to the fourth decimal and provided with a thermometer indicating tenths of a degree. The temperature at which the specific gravity is taken should be that for which the hydrometer has been constructed, the urine being heated or cooled to the desired degree. One hundred to 200 c.c. are then set aside in a flask, after the addition of some yeast which has been washed free from mineral material, loosely stoppered or provided with an arrangement like the one shown in the accompanying figure (Fig. 114). After twenty-four hours if but little sugar is present, or forty-eight hours if there is much, the specific gravity is again determined under the precautions given, after having filtered the urine. The difference in the specific gravity is multiplied by 230, an empirical factor which has been found by dividing the amount of sugar ascertained by titration or polarization with the difference in the density of the urine after fermentation. The result indicates the percentage of sugar.

The process may be hastened if to each 100 c.c. of urine 2 grammes of potassium and sodium tartrate and 2 grammes of diacid-sodium phosphate are added, with 10 grammes of compressed yeast, and the mixture is kept at a temperature of from 30° to 34° C. If but little sugar is present, two to three hours will be sufficient.

That portion of the urine of which the specific gravity is determined before fermentation should really be treated in the same manner. It will suffice, however, to add 0.022 to the specific gravity found, to make up for the increase that would otherwise be observed in the second specimen owing to addition of the salts.

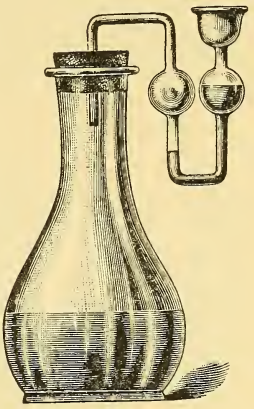
In every case the urine must be perfectly fresh, as fermentation generally begins spontaneously, even after standing a short time.

Einhorn's Method.—This will answer very well for ordinary purposes. Two especially constructed and graduated saccharimetric tubes (Fig. 113) are used, one of which is filled with a mixture of the suspected urine and yeast, and the other with normal urine and

[1] Roberts, Lancet, 1862, i. p. 21. Worm-Müller, Pflüger's Archiv, 1884, vol. xxxiii. p. 211, and 1885, vol. xxxvii. p. 479.

yeast, as a control. The tubes are set aside at a temperature of from 30° to 34° C., when the percentage-amount of sugar in the urine is read off from the column of carbon dioxide formed. Should the second tube also show a small amount of gas, the figure corresponding to this amount is deducted from the first.

Fig. 114.



Flask for the approximate estimation of sugar by fermentation. (v. Jaksch.)

Lohnstein's Method.—A very convenient modification of Einhorn's instrument, and one furnishing more accurate results, has been introduced by Lohnstein.[1] As will be seen from the accompanying figure (Fig. 115), this is essentially a U-tube open at both ends. The longer limb is closed during the process of fermentation by a ground-glass stopper. This stopper is provided with an air-hole, to which a similar hole corresponds in the drawn-out portion of the tube. The apparatus is filled with the urine to be examined, through the bulb *A*, while the two air-holes at *B* are in communication. Care should be had that the liquid stands exactly at the mark 0. The stopper is then turned so that all communication between the air and the urine is cut off. A little mercury is finally poured into the saccharimeter, when the instrument is placed in a vessel containing water at 35°–40° C., and maintained at a temperature of about 30° C. After twelve hours the percentage of sugar is read off directly.

Precautions: 1. As every urine contains traces of free carbon dioxide, it is well to remove this by boiling if we have reason to suppose that only a small amount of sugar is present. Before adding the yeast the urine is, of course, cooled to the surrounding temperature.

2. As the instrument yields satisfactory results only if the urine contains less than 1 per cent. of sugar, it is necessary to dilute it with water when more is present. The specific gravity may here serve as an index; urines of a specific gravity up to 1.018 are examined directly; from 1.018 to 1.022 they are diluted twice, from 1.022 to 1.028 five times, and those above 1.028 ten times.

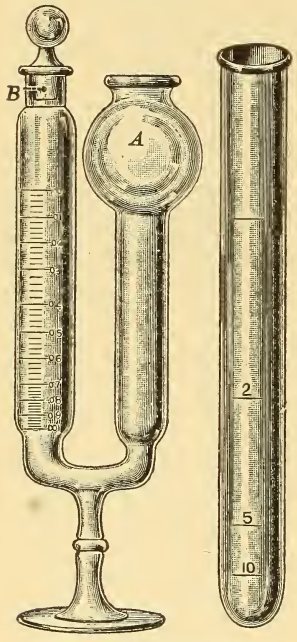
3. A test-tube, provided with the necessary marks to indicate the degree of dilution of the urine, accompanies the instrument. In every case a globule of yeast, approximately 6–8 mm. in diameter,

[1] T. Lohnstein, "Ein neues Gährungssaccharometer," Berlin. klin. Woch., 1898, p. 866.

is added to the urine and shaken in the tube until an even suspension has been reached.[1]

Polarimetric Method.—For this purpose the saccharimeter of Soleil-Ventzke is very convenient (Fig. 116). This consists essentially of a Nicol prism, A, which may be rotated about the axis of the apparatus; a second Nicol prism, at D; vertically placed compensating prisms, consisting of dextrorotatory quartz, at E, which may be moved horizontally by means of a rack-and-pinion adjustment, turned by a milled head at K, so that light can pass through a thicker or thinner layer of the dextrorotatory quartz. At F is a plate of lævorotatory quartz cut perpendicularly to the optical axis, and covering the entire field of vision; at H biquartz plates of Soleil, and at I an Iceland-spar crystal; BC represents a small telescope, by means of which the biquartz plates can be accurately focussed. When the compensation-prisms of this apparatus are in a certain position the lævorotation of the plate F will be exactly compensated, and the two halves of the field of vision present the same color, while the zero of the scale X will coincide with the zero of the vernier Y, arranged on the upper surface of the compensators. Any change in this position produced by turning the screw K will cause the appearance of a different color in each half of the field of vision. If now, with a zero-position, an optically active dextrorotatory or lævorotatory substance is interposed, the color of each half of the field of vision will become altered, but may be equalized again by changing the position of the compensators, the degree of change necessary to produce this result constituting an index of the power of rotation of the solution interposed in the tube M.

Fig. 115.



Lohnstein's saccharimeter.

Soleil-Ventzke's apparatus is constructed in such a manner that if a solution of glucose is employed, the length of the tube M being 10 cm., every entire line of division on the scale will indicate 1 per cent. of sugar.

The tube of the saccharimeter should be carefully washed out with distilled water, and at least once or twice with the filtered urine, when it is placed on end upon a flat surface and filled with the

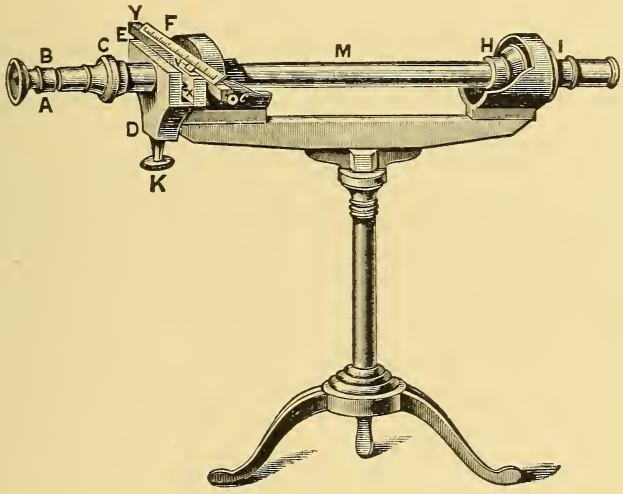
[1] Lohnstein's saccharimeter may be procured from R. Kallmeyer & Co., Oanreinburger Str. 45, Berlin.

urine, so that this forms a convex cup at the end. The glass plate is now carefully adjusted, so as to guard against the admission of bubbles of air. The metallic cap is placed in position, care being taken to avoid undue pressure. The examinations are made in a dark room; an ordinary lamp is used, and several readings are taken, until the differences do not amount to more than 0.1 or 0.2 per cent. The tubes should be thoroughly cleansed *immediately* after the experiment.

In every case the filtered urine should be free from albumin, and, if markedly colored, should be previously treated with neutral lead acetate in substance and filtered.

If it is desired to demonstrate only the presence of sugar, the compensators are first brought to the zero-position. If now, upon

Fig. 116.



Soleil-Ventzke's saccharimeter.

interposition of the tube filled with urine, a difference in the color of the two halves of the field of vision is noted, the presence of an optically active substance in the urine may be assumed; and if the deviation is at the same time to the right, the presence of glucose is rendered highly probable, while a deviation to the left will generally be referable to levulose or β-oxybutyric acid. Indican, peptones (albumoses), cholesterin, and certain alkaloids, it is true, also turn the plane of polarization to the left; but as a rule these substances need not be considered, as cholesterin occurs but rarely, and indican is usually present in only small amounts in diabetic urines. Albumoses, if present, must first be removed. Lactose and maltose, which also turn the plane of polarization to the right, may be dis-

tinguished from each other and from glucose by the phenylhydrazin test. Levulose turns the plane of polarization to the left. Oxybutyric acid is practically always associated with the presence of glucose, and may be recognized by allowing the urine to undergo fermentation, when the filtered urine will become distinctly lævorotatory.

Bremer's Diabetic Urine Test.[1]—The test is based upon the different behavior toward certain anilin dyes of diabetic, as compared with non-diabetic, urine. If a trace of a mixture of 2 parts of eosin and 3 parts of gentian-violet, for example, is added to non-diabetic urine, it will be observed that the urine gradually dissolves the eosin and assumes a yellowish or bright-red color, while the gentian-violet fails to dissolve. If diabetic urine, on the other hand, is treated in the same manner, the eosin will likewise dissolve, but a solution of the gentian-violet also occurs, and the entire specimen eventually assumes a violet color.

Of late, Bremer has advised the use of Merck's gentian-violet B, or of methyl-violet 5B. The test is extremely simple : two well-dried test-tubes are filled to about one-half, the one with normal urine and the other with the urine to be examined. About 0.5 mgrm. of either of the above reagents is then placed upon the surface of the urine ; the tubes are kept in a warm place or immersed in warm water. On standing, streaks of blue gradually appear in both specimens, but on shaking the color disappears in the normal specimen, while the entire bulk of the diabetic urine assumes a blue or violet color. A reddish-purplish color is often observed in non-diabetic specimens, but is of no significance. Bremer admits that doubtful results may be obtained with urines presenting a specific gravity below 1.014 or 1.015, and that in such cases it may be impossible to distinguish non-diabetic from diabetic urine. He claims, on the other hand, that a positive result with a urine of high specific gravity is pathognomonic of diabetes, and that this may be obtained even at a time when the sugar has temporarily disappeared from the urine.

The substance which gives rise to this peculiar reaction is unknown. Sugar in itself, as also acetone and diacetic acid, are not concerned in its production. The reaction of the urine also is unimportant. Bremer is inclined to believe that in non-diabetic urines one of the coloring principles helps to render the urine refractory. As he says, colorless diabetic urines yield the most striking color-reactions, and especially those in which a greenish shimmer is apparent.

On the whole, Bremer's observations have been confirmed so far

[1] L. Bremer, "Anilinfarbenproben d. Harns bei Diabetes," Centralbl. f. inn. Med., vol. xix. p. 307. T. B. Futcher, Phila. Med. Jour., 1898. L. Bremer, "On the Chemical Behavior of Eosin and Gentian-violet toward Normal and Diabetic Urines," N. Y. Med. Jour., 1897.

as diabetic urine is concerned. Exceptions, however, occasionally occur even in cases of true diabetes, and, as Bremer admits, positive results are frequently observed in urines of a low specific gravity.

The test is of interest, and may possibly be further modified so as to be of value in diagnosis, but as yet it would scarcely be warrantable to draw definite conclusions from its occurrence, even when the specific gravity is high.

Lactose.—Lactose may be found in the urine toward the end of gestation, but it occurs more especially in nursing-women in whom the flow of milk is impeded. It is generally stated, however, that lactosuria also occurs in nursing-women who have well-developed breasts, in the absence of any obstruction, and that the *good qualities* of a wet-nurse are indicated by a copious and persistent elimination of milk-sugar. Its presence may be inferred if a positive result is obtained with Trommer's and Nylander's tests, while the phenylhydrazin and fermentation tests give negative results, although an osazon can be obtained from the *isolated* substance, and although lactose undergoes a certain form of alcoholic fermentation.

Lemaire, who has recently investigated this subject, found that the urine of nineteen women examined in this direction apparently contained no sugar during the last twelve days preceding confinement (Trommer's and Nylander's tests), while a positive reaction was obtained with Trommer's reagent in two cases and with Nylander's reagent in thirteen cases after confinement. The phenylhydrazin test was negative in all nineteen before and positive after confinement, *when this was directly applied to the substance isolated according to Baumann's method.* The percentage varied between 0.013 and 0.438, and appeared to be uninfluenced by the act of nursing.[1]

Lævulose.[2]—It is claimed that lævulose is occasionally found in diabetic urines together with glucose, and may also occur spontaneously unaccompanied by glucosuria. Such urines show a deviation to the left or none at all, while the other tests for sugar indicate the presence of a reducing substance.

Maltose.—Maltose, together with glucose, was first found in the urine of a patient supposedly the subject of pancreatic disease, associated with an acholic condition of the stools. Since that time it has been repeatedly observed in diabetic patients. In one case the amount was 27.8 grammes pro liter. Similar results have been obtained in dogs after extirpation of the pancreas.[3] Its recognition is practically dependent upon the formation of its osazon and a determination of the melting-point of the latter. Such urines, moreover,

[1] De Sinety, Maly's Jahresber., 1874, vol. iii. p. 134. Hempel, Arch. f. Gynaek., 1875, vol. viii. p. 312. Ney, Ibid., 1889, vol. xxxv. p. 239. F. Hofmeister, "Ueber Laktosurie," Zeit. f. physiol. Chem., 1877, vol. i. p. 101 (lit.). F. A. Lemaire, Ibid., 1896, vol. xxi. p. 442.

[2] Seegen, Centralbl. f. d. med. Wiss., 1884, vol. xxii. p. 753. H. Rosin and L. Labaud, Zeit. f. klin. Med., vol. xlvii. Hefte 1 u. 2.

[3] Lépine and Boulud, Compt. rend., vol. cxxxii. p. 610.

show a larger percentage of sugar on polarization than on titration with Fehling's solution. At the same time it will be observed that on heating for two hours with hydrochloric acid at 106° F. the polarimetric values become smaller, while the titration values increase.

Dextrin.[1]—In one case of diabetes dextrin appeared to take the place of glucose. It may be recognized by the fact that upon the application of Fehling's test the blue liquid first becomes green, then yellow, and sometimes dark brown. Traces of dextrin are probably present in every urine, but cannot be demonstrated with the common tests.

Laiose.[2]—Laiose has been found in the urine of a diabetic patient. It is essentially characterized by the fact that on titration with Fehling's solution from 1.2 to 1.8 per cent. more sugar is indicated than by the polarimetric method.

Pentoses.—Traces of pentoses, viz., xylose, arabinose, and rhamnose, may be found in every urine. Larger quantities were first observed by Salkowski and Jastrowitz, in the urine of a morphine *habitué*, in which the pentosuria alternated with glucosuria. A similar case was reported by Real. Külz and Vogel found larger quantities in a case of diabetes; and still more recently Bial has reported two instances which occurred in apparently healthy individuals. A digestive pentosuria has also been described. Such urines reduce Fehling's solution and Nylander's solution, and give rise to the formation of an osazon when treated with phenylhydrazin. The osazon, however, can be readily distinguished from that obtained from glucose, maltose, or lactose, etc., by the melting-point (159°–160° C.). The fermentation test is negative. Xylose and rhamnose turn the plane of polarization to the right, while arabinose is optically inactive. The presence of pentoses can be definitely established with Tollens' orcin test:

Tollens' Orcin Test.—A few granules of orcin are dissolved in 4 to 5 c.c. of concentrated hydrochloric acid by the aid of heat, so that a slight excess is present. This solution is divided into two equal parts and allowed to cool. To one portion 0.5 c.c. of the urine to be examined is added, and to the other an equal amount of normal urine of the same specific gravity. Both specimens are placed in a beaker containing boiling water, when in the presence of pentoses a green color will first be observed at the top, which gradually extends throughout the mixture, while the normal specimen scarcely changes in color. In the presence of 0.1 per cent. a positive reaction is still obtained, which is especially marked if the urine has been previously decolorized with animal charcoal. The green pigment which results can be extracted by shaking with

[1] Reichard, Maly's Jahresber., 1876, vol. v. p. 60.
[2] Leo, Virchow's Archiv, vol. cvii.

amyl alcohol, and on spectroscopic examination it gives rise to a well-defined band of absorption in the red portion of the spectrum near the yellow border.

Tollens' phloroglucin test, in which phloroglucin is substituted for the orcin, and in which a deep-red color is obtained in the presence of a pentose, may also be used, but the reagent indicates the presence of glucuronates as well.

Very curiously, the pentosuria persists even though no carbohydrates are ingested; and there is evidence to show that pentoses are formed within the body. As a matter of fact, Hammarsten has succeeded in demonstrating the presence of a pentose among the decomposition-products of a nucleo-glucoproteid which is found in the pancreas; and Blumenthal arrived at similar results in the case of various nucleinic acids which occur in the animal body. It is possible, on the other hand, that the pentoses may result from the metabolic products of glucose which are formed under normal conditions by a process of oxidation, and are then eliminated as such under still unknown influences.

Aside from the traces normally present in the urine, pentosuria must be regarded as a metabolic anomaly, analogous to glucosuria, cystinuria, alkaptonuria, etc.

LITERATURE.—E. Salkowski u. M. Jastrowitz, "Ueber eine bisher nicht beobachtete Zuckerart im Harn," Centralbl. f. d. med. Wiss., 1892, No. 19. E. Salkowski, "Ueber d. Pentosurie," Berlin. klin. Woch., 1895, No. 17. F. Blumenthal, Ibid., No. 26; and Zeit. f. klin. Med., vol. xxxvii. p. 415. E. Külz u. J. Vogel, Zeit. f. Biol., N. F., 1896, vol. xiv. p. 189. E. Salkowski, "Ueber d. Vorkommen von Pentosen im Harn," Zeit. f. physiol. Chem., 1899, vol. xxvii. p. 587. Bial, Ueber Pentosurie, Zeit. f. klin. Med., 1900, vol. xxxix. p. 472.

Animal Gum.—Landwehr's animal gum, according to modern researches, is a constant constituent of normal urine, but is of no clinical interest. Of the chemical nature of the substance not much is known, but there is evidence to show that in all probability the body is a derivative of chondroitin-sulphuric acid.

GLUCURONIC ACID.

Glucuronic acid is derived from glucose, and constitutes an intermediary product of the normal metabolism of the body. In the urine it is found only in combination with certain fatty and aromatic alcohols, forming compounds which are related to the glucosides and are generally spoken of as the *conjugate glucuronates.* Such bodies have been observed in the urine following the ingestion of chloral, camphor, naphtol, oil of turpentine, menthol, phenol, morphin, antipyrin, etc., and traces may also be obtained from normal urines. The normal glucuronates are undoubtedly compounds of glucuronic acid with phenol, paracresol, indoxyl, and skatoxyl.

Their amount is exceedingly small, as the greater portion of these bodies is normally eliminated in combination with sulphuric acid.

Of the quantitative variations of the normal glucuronates and their relation to disease, next to nothing is known. Their clinical interest centres in the fact that certain glucuronates are capable of reducing copper and bismuth in alkaline solution, and may thus be confounded with glucose. Such urines, however, do not undergo fermentation. The glucuronates turn the plane of polarization to the left, while glucuronic acid itself is dextrorotatory. Like the pentoses, the glucuronates give a positive reaction with phloroglucin, while they do not react with orcin (see page 532). With the free acid phenylhydrazin forms crystalline compounds (see page 520).

LITERATURE.—H. Thierfelder, "Ueber d. Bildung v. Glykuronsäure," etc., Zeit. f. physiol. Chem., 1886, vol. x. p. 163; "Untersuchungen über d. Glykuronsäure," Ibid., 1887, vol. xi. p. 388. P. Mayer, "Ueber d. Ausscheidung u. d. Nachweis d. Glykuronsäure," Berlin. klin. Woch., 1899, pp. 591 and 617. P. Mayer u. C. Neuberg, Zeit. f. physiol. Chem., 1900, vol. xxix. p. 256.

INOSIT.

According to Hoppe-Seyler, traces of inosit may be found in the urine under normal conditions. Somewhat larger quantities are eliminated following the ingestion of large amounts of water, and for this reason possibly inosituria is notably observed in cases of diabetes insipidus, in diabetes mellitus, and in chronic interstitial nephritis. Its occurrence in these diseases is, however, not constant. The substance is devoid of clinical interest. It is not a carbohydrate, but belongs to the aromatic series, and is commonly regarded as hexa-hydroxybenzol. Its formula is $C_6H_{12}O_6 + H_2O$. For methods of isolating the substance from the urine, the reader is referred to special works.[1]

URINARY PIGMENTS AND CHROMOGENS.

Under normal conditions urochrome and uroerythrin, to which latter the red color of urate sediments is due, are the only known pigments which occur preformed in the urine, while indigo-red and indigo-blue, derived from indoxyl sulphate and indoxyl glucuronate, may be artificially produced. In disease, on the other hand, various other pigments may be found, which occur in the urine either free or in the form of chromogens. Among the former may be mentioned hæmoglobin, methæmoglobin, hæmatin, hæmatoporphyrin, urorubrohæmatin, urofuscohæmatin, urobilin, the biliary pigments, and melanin; while abnormal chromogens are met with following the ingestion of certain drugs, such as santonin, senna, rheum, iodine, etc., as also in cases of poisoning with carbolic acid, creosote, etc. The occurrence

[1] C. E. Simon, Physiological Chemistry, Lea Bros. & Co.

of some of these substances, such as the various forms of blood-pigment, the biliary pigments, and indigo, viz., indican, is of considerable clinical interest, while others again are of only minor importance.

Normal Pigments.—**Urochrome.**—To the presence of this pigment, which appears to be identical with the *normal urobilin of MacMunn*, but which should not be confounded with the *pathological urobilin of Jaffé*, the normal yellow color of the urine is probably largely due. It is supposedly derived from bilirubin, which in turn is referable to hæmatin, and thus to the hæmoglobin of the blood. From the bilirubin secreted into the intestinal tract it is derived by a process of oxidation, and not of reduction, as is generally stated (Gautier). Such a transformation, according to our present knowledge, may, however, also occur directly, without the intervention of bilirubin, as urochrome is found in the urine of dogs in which the bile is prevented from entering the intestinal tract by the establishment of a biliary fistula. An increased amount is similarly found in cases in which resorption of large extravasations of blood is taking place—in short, whenever an increased destruction of red corpuscles occurs. Under the opposite circumstances—*i. e.*, in conditions associated with a new formation of red corpuscles, as in certain forms of anæmia, chronic parenchymatous nephritis, diabetes, diseases of the bone-marrow, etc.—it occurs in diminished amount. Urochrome, moreover, is present in urobilin-free feces, and even in those of infants with congenital atresia of the biliary ducts.

In order to obtain urochrome from normal urine, this is acidulated with 1–2 grammes of dilute sulphuric acid pro liter, filtered, and saturated with ammonium sulphate in substance, when the flakes which are formed, if an excess of the salt has been added, are dried and treated with warm, slightly ammoniacal absolute alcohol; the pigment is then obtained upon evaporation of the alcohol.

An alcoholic solution of urochrome, like the urobilin of Jaffé, is said to exhibit a beautiful greenish fluorescence when treated with ammonia and a few drops of a solution of zinc chloride; but, unlike the latter substance, its acidulated alcoholic solutions present a broad band of absorption at *F*, which extends more to the left than to the right of this line, while the remainder of the spectrum is at the same time absorbed to the right end, from a point somewhat to the left of *G*. Garrod, on the other hand, states that by acting upon urochrome with acids he did not succeed in obtaining any product showing the urobilin band or yielding the well-known fluorescence with zinc chloride and ammonia. But a substance having both these properties was readily obtained by the action of aldehyde upon an alcoholic solution of the pigment. In a short time—shorter still when the liquid is warmed—an absorption-band appears like that of urobilin, and the tint of the solution deepens to a rich orange-yellow. With zinc chloride and ammonia a brilliant green fluorescence appears, and the band is shifted toward the

red, as that of urobilin is under like circumstances. The process can be stopped at this point by the simple addition of water, for aldehyde has no such action upon aqueous solutions of urochrome. If, however, the action be allowed to continue, a further change ensues; the liquid reddens, and a second band appears in the violet. The fluorescence can still be obtained with zinc chloride and ammonia, and both bands are shifted toward the red and are closer together than before. The reaction with aldehyde, according to Garrod, affords a very delicate test for the presence of urochrome in alcoholic solutions. The product of the earlier stage, although it is not identical with urobilin, resembles that pigment quite as closely as the products obtained from bilirubin and hæmatin by the action of reducing agents; but no second band is developed when aldehyde is added to an alcoholic solution of urobilin.[1]

By the action of potassium permanganate upon urobilin Riva and Chiodera[2] obtained a substance closely resembling urochrome, and a similar product is formed when an aqueous solution of urobilin containing ether is evaporated upon a water-bath. Neither product shows any absorption-band, and both behave as urochrome does when it is acted upon by aldehyde.

Uroerythrin.—Uroerythrin is the pigment which imparts the red color to crystals of uric acid and the pink tint to urate sediments. Under strictly normal conditions it probably does not occur in the urine, but it readily appears with the slightest deviation from health, and when present in larger amounts imparts a deep-orange color to the urine. Under pathological conditions it is seen especially in cases of hepatic insufficiency, in which the liver, owing to a greatly increased destruction of red corpuscles, is unable to transform into bile-pigment all the blood-pigment which is carried to it. It also occurs when an absolute insufficiency on the part of the hepatic cells exists, so that the organ is not even capable of causing the transformation of a *normal* amount of hæmoglobin. Uroerythrin is thus seen in notable quantities in cases of cirrhosis and carcinoma of the liver, in passive congestion resulting from heart-disease, in acute articular rheumatism, gout, pneumonia, malarial fever, erysipelas, spinal curvature, etc. In typhoid fever a marked excretion of uroerythrin is exceptional, and its occurrence has been associated with pulmonary complications. In nephritis it is seldom found in the urine, but Garrod cites an instance of pneumonia in which an abundant excretion of the substance accompanied conspicuous albuminuria.

In certain diseases, such as hepatic cirrhosis, the excretion of uroerythrin, as also of urobilin, is said to be much diminished when the patient is placed upon a milk-diet (Riva).

[1] A. E. Garrod, "The Bradshaw Lecture on the Urinary Pigments in Their Pathological Aspects," Lancet, Nov. 10, 1900.

[2] Riva and Chiodera, Arch. ital. di Clin. Med., 1896, vol. xxxv. p. 505.

Chemically, its relation to hæmoglobin, hæmatoidin, and bilirubin is seen from the following analyses of the various pigments:

	C	H	N	O	S	Fe
Hæmoglobin,	53.85	7.32	16.17	. . .	0.39	0.43
Hæmatoidin,	65.05	6.37	9.51	. . .	. .	. .
Bilirubin,	67.83	6.29	9.79	16.79	. .	. .
Uroerythrin,	62.51	5.79	31.70		. .	. .

When present in large amounts uroerythrin is readily recognized by the salmon-red color which it imparts to urinary sediments. Otherwise it is best to precipitate the urine with neutral lead acetate, barium chloride, or a similar reagent, when in the absence of uroerythrin a milky-white precipitate is obtained, while a pale rose-colored sediment indicates the presence of the pigment in appreciable amounts; a more pronounced rose color is produced if large quantities are present. In every case at least ten to fifteen minutes should be allowed to elapse before forming a definite conclusion, so that the sediment may have abundant time to settle.

The pigment itself is unstable. Its solutions in alcohol or chloroform are rapidly decolorized by light, and even when kept in the dark quickly undergo change. Alkalies destroy the pigment readily, with the production of a green tint. Neutralization of the alkali does not restore the original color or bring back the absorption spectrum, which is characteristic, though ill-defined, consisting of two faint bands in green and blue, united by a fainter shading. One of these bands has the position of the urobilin band, but both alike disappear when the solutions are decolorized by light. The pigment is readily soluble in amyl alcohol and acetic ether (Garrod).[1]

Normal Chromogens.—The chromogens occurring in normal urine are indican, urohæmatin, and an unknown chromogen which yields uroroseïn when treated with mineral acids.

Indican.—It has been pointed out (see Sulphates) that the indol formed during intestinal putrefaction is oxidized to indoxyl in the blood; this, entering into combination with sulphuric acid, is eliminated in the urine as sodium or potassium indoxyl sulphate, or indican, as represented by the equations:

(1) $C_8H_7N + O = C_8H_7NO$

Indol. Indoxyl.

(2) $C_8H_7NO + SO_2(OH)(OH) = SO_2(C_8H_6NO)(OH) + H_2O$

Indoxyl. Indoxyl suiphate.

(3) $SO_2(C_8H_6NO)(OH) + Na_2HPO_4 = SO_2(C_8H_6NO)(ONa) + NaH_2PO_4$

Indoxyl sulphate. Indoxyl-sodium sulphate.

[1] A. E. Garrod, loc. cit. A. Robin, Urologie clinique de la Fièvre typhoïde, Paris, 1877.

Formerly it was thought that indican was also formed within the tissues of the body in the absence of putrefactive organisms.[1] Further researches, however, have demonstrated that micro-organisms are always concerned in the production of indican, and that in health the large intestine is its sole source. Baumann, who succeeded in absolutely disinfecting the intestinal tract of a dog by means of large doses of calomel, thus observed that all traces of indican, as also of phenol and paracresol, disappeared from the urine. According to Senator, moreover, indican does not occur in the urine of newly born infants which have not as yet received nourishment. This observation is a strong point in favor of Nencki's teachings that indol is a specific product of albuminous putrefaction in the presence of organized ferments, as putrefiable substances are here present, but no putrefactive organisms. Tuczek's observations on abstinence from food in cases of insanity, in which indican was observed in the urine only when albumins, though in minimal amounts, were ingested, also speak very strongly against Salkowski's theory. Finally, it has been demonstrated that in cases in which an artificial anus is established near the distal end of the ileum the conjugate sulphates disappear almost entirely from the urine, while they reappear in normal amount as soon as the connection between the small and large intestines has been re-established.[2]

The amount of indican which is normally eliminated in the urine varies somewhat with the character of the diet. Jaffé[3] obtained 6.6 mgrms. from 1000 c.c. of urine, as an average of eight observations. The largest quantities excreted in health are found after a liberal indulgence in animal food, particularly the so-called red meats, while the smallest amounts are observed during a milk- or kefir-diet. By means of the latter article, indeed, the greatest diminution in the degree of intestinal putrefaction may be effected in man.

In pathological conditions an increased elimination of indican is observed:

1. In all diseases which are associated with an increased degree of intestinal putrefaction. As there appears to be little doubt that this is largely regulated by the acidity of the gastric juice, an increased indicanuria, according to personal observations, is encountered when anachlorhydria or hypochlorhydria exists. It has been pointed out elsewhere that it is possible to form a fairly accurate idea of the amount of free hydrochloric acid in the gastric juice by an examina-

[1] E. Salkowski, Ber. d. deutsch. chem. Ges., 1876, vol. ix. pp. 138 and 408. Baumann, Zeit. f. physiol. Chem., 1886, vol. x. p. 123. Senator, Centralbl. f. d. med. Wiss., 1877, vol. xv. pp. 357, 370, and 388.

[2] Nencki, Macfadyen u. Sieber, Arch. f. exper. Path. u. Pharmakol., 1891, vol. xxix.

[3] Jaffé, Centralbl. f. d. med. Wiss., 1872, vol. x. pp. 2, 481, and 497; and Virchow's Archiv, 1877, vol. lxx. p. 72.

tion of the urine in this direction. Large quantities of indican are thus eliminated in cases of carcinoma of the stomach, and exceeded only by those observed in cases of ileus, so that this symptom, in my estimation, is of considerable value in differential diagnosis, and is one, moreover, which has not received the attention it deserves. Exceptions to this rule are at times, though rarely, met with, for which it is, however, impossible to account at present. Large quantities of indican are also observed in cases of acute, subacute, and chronic gastritis. In the course of personal observations in this direction I was impressed with the curious phenomenon that in cases of ulcer of the stomach, notwithstanding the simultaneous occurrence of hyperchlorhydria, an increased elimination of indican, contrary to what is usually seen in hyperchlorhydria referable to other causes, is quite constantly found. Possibly the existence of muscular atony which was noted in those cases may serve to explain this apparent incongruity, but it is as yet impossible to offer a satisfactory explanation of the phenomenon. Remembering the origin of indican, and the relation which the amount eliminated bears to the degree of intestinal putrefaction, it will be unnecessary to enumerate the long list of diseases in which an increased indicanuria has been observed, as it will be found that in the majority of these cases the indicanuria is merely an index of the condition of the gastric juice and the motor power of the stomach.[1]

2. It should be noted that in cases in which the peristaltic movements of the *small* intestine have become impeded, as in ileus, acute and chronic peritonitis, an increased elimination of indican will invariably take place, no matter what the state of the gastric juice may be. In such conditions, and especially in ileus, the largest quantities are observed, a point which may be of *decided* value in differential diagnosis, as diseases of the large intestine alone are *never* associated with an increase in the amount of indican. *In simple, uncomplicated constipation increased indicanuria is not seen;* and should an examination in such cases reveal the presence of more indican than normal, it will be safe to assume the existence of disease elsewhere, and especially of the stomach.

3. As albuminous putrefaction may also take place within the body, an increased indicanuria is observed in cases of empyema, putrid bronchitis, gangrene of the lung, etc.; but while in the conditions mentioned above the indol-producing organisms appear to be especially active, the elimination of phenol in the latter condition may be more pronounced at times than that of indican. Bearing in mind the points here set forth, I cannot agree with others in saying that the study of indicanuria possesses no importance from a clini-

[1] C. E. Simon, "Indicanuria," Am. Jour. Med. Sci. (full literature), 1895, vol. cx. p. 48.

cal standpoint. I maintain, on the other hand, that *an examination of the urine in this direction is at least as important as the testing for albumin and sugar, and that points of decided importance, not only in diagnosis, but also in prognosis and treatment, may thus be gained.*

Of interest in this connection also is the observation that in cases of increased indicanuria oxalate sediments are not uncommonly observed; but I am not willing to admit, as Harnack and van der Leyen suggest, that the indicanuria which follows the ingestion of small doses of oxalic acid is produced by a toxic action of the acid upon the tissue albumins. In these cases also the increased indicanuria is referable to increased intestinal putrefaction.[1]

When indican is treated with hydrochloric acid, it is decomposed into sulphuric acid and indoxyl; should an oxidizing substance be present at the same time, indigo-blue, the blue coloring-matter of the urine, results:

$$\underset{\text{Potassium indoxyl sulphate.}}{2C_8H_6NKSO_4} + 2O = \underset{\text{Indigo-blue.}}{C_{16}H_{10}N_2O_2} + 2HKSO_4.$$

Indigo-blue in small amounts may be found free in the sediment of almost every decomposing urine, usually occurring in the form of small, amorphous granules, and more rarely in crystalline form. Urines have, however, also been observed which were blue when passed, or which turned blue as a whole upon standing. Such a phenomenon must be regarded as a medical curiosity. Undoubtedly it is referable to the action of micro-organisms (see page 581), although McPhedran and Goldie mention that in their case bacteria were present only in small numbers.[2]

The blue pigment which may be obtained from urines has been variously described as Prussian-blue, urocyanin, cyanurin, Harnblau, uroglaucin, choleraic urocyanin, but it has been shown to be indigo-blue, and derived from a colorless mother-substance which is present in every urine to a greater or less extent, and which has been named indican. This has been shown to be identical with the uroxanthin of Heller and Thudichum's choleraic urocyaninogen.

Tests for Indican.—The urine of twenty-four hours is carefully collected and a specimen taken for examination. A few cubic centimeters are then mixed with an equal volume of Obermayer's reagent, and shaken with a small amount of chloroform. *Obermayer's reagent* is a 2 pro mille solution of ferric chloride in concentrated hydrochloric acid.[3]

[1] v. Moraczewski, "Oxalurie and Indicanurie," Cent. f. inn. Med., 1903, No. 1.

[2] A. McPhedran and W. Goldie, "A Case of Indigosuria," Trans. Assoc. Am. Phys., 1901, vol. xvi. p. 242.

[3] Obermayer, Wien. klin. Woch., 1890, vol. iii. p. 176.

Stokvis' modification of Jaffé's test may also be employed.[1] To this end, a few cubic centimeters of urine are treated with an equal volume of concentrated hydrochloric acid, and two or three drops of a strong solution of sodium or calcium hypochlorite. The mixture is shaken with 1 or 2 c.c. of chloroform as above. The indigo which is set free in this manner is taken up by the chloroform, and colors this blue to a greater or less extent, the degree of increase, as compared with the normal, being determined by the intensity of the color. Albumin need not be removed. Bile-pigment, which interferes with the reaction, is removed by means of a solution of lead subacetate, which is carefully added in order to avoid an excess. Urines presenting a very dark color may be cleared in the same manner. Potassium iodide, owing to the liberation of free iodine, will color the chloroform more or less of a carmine. For the sake of comparison, it is well to employ the same quantities of urine and reagents in every case, marked tubes being very convenient for this purpose.

The method last described I have also found to be a fairly sensitive test for albumin, in the presence of which a well-marked cloud appears near the surface of the mixture and gradually extends downward.

Quantitative Estimation.—*Wang's Method.*[2]—The method is based upon the decomposition of potassium indoxyl sulphate by means of concentrated hydrochloric acid and the oxidation to indigo-blue of the indoxyl which is thus formed. The indigo-blue is further transformed into indigo-sulphuric acid, and this titrated with a solution of potassium permanganate of known strength. The various changes which take place are represented by the following equations :

(1) $\underset{\text{Indican.}}{C_8H_6NSO_4K} + H_2O = \underset{\text{Indoxyl.}}{C_8H_6N.OH} + HKSO_4.$

(2) $2\underset{\text{Indoxyl.}}{C_8H_6N.OH} + 2O = \underset{\text{Indigo-blue.}}{C_{16}H_{10}N_2O_2} + 2H_2O.$

(3) $\underset{\text{Indigo-blue.}}{C_{16}H_{10}N_2O_2} + 2H_2SO_4 = \underset{\text{Indigo-sulphuric acid.}}{C_{16}H_8(HSO_3)_2N_2O_2} + 2H_2O.$

(4) $\underset{\text{Indigo-blue.}}{5C_{16}H_{10}N_2O_2} + 4KMnO_4 + 6H_2SO_4 = 5C_{16}H_{10}N_2O_4 + 2K_2SO_4 + 4MnSO_4 + 6H_2O.$

Reagents required : 1. A 20 per cent. solution of lead acetate.

2. Obermayer's reagent. This is a 2 pro mille solution of ferric chloride in concentrated hydrochloric acid (sp. gr. 1.19).

3. Chloroform.

4. Concentrated sulphuric acid.

[1] See Senator, Centralbl. f. d. med. Wiss., 1877, vol. xv. p. 257.

[2] E. Wang, "Ueber d. quantitative Bestimmung d. Harnindikans," Zeit. f. physiol. Chem., vol. xxv. p. 406.

5. A mixture of equal parts of alcohol (96 per cent.), ether, and water.

6. A concentrated solution of potassium permanganate—*i. e.*, a solution containing about 3 grammes pro liter. The titration is conducted with this solution diluted in the proportion of 5 c.c. to 195 c.c. of water. Its titre is ascertained before each titration by comparing it with a dilute solution of oxalic acid of known strength; for example, one containing 0.1 gramme of the acid dissolved in 100 c.c. of water, as described on page 450. The amount of indigo-blue which each cubic centimeter will represent is ascertained by multiplying the corresponding amount of oxalic acid by 1.04.

Example.—Supposing that the permanganate solution is found of such strength that 1 c.c. represents 0.00014 gramme of oxalic acid; the corresponding amount of indigo would be $0.00014 \times 1.04 = 0.00015$ gramme.

Method.—The urine is first examined for indican, as described above. Should a very intense reaction be thus obtained, only 25 or 50 c.c. are used for the quantitative estimation, while larger amounts are taken (200–500 c.c.) if the reaction is of only moderate intensity or negative altogether.

The urine is precipitated with lead acetate solution, care being taken to avoid an excess. A large and accurately measured portion of the clear filtrate is treated in a separating funnel with an equal volume of Obermayer's reagent and extracted with chloroform. To this end, 30 c.c. are added at a time and shaken for one minute. Two or three extractions are usually sufficient to remove the entire amount of indigo. The extract is placed in a small flask, and the chloroform distilled off. The residue is dried for a few minutes on a water-bath until traces of remaining chloroform have been removed. It is then washed with the alcohol-ether-water mixture to remove the reddish-brown pigment which is present together with the indigo-blue. The latter remains undissolved. After filtering off any particles of indigo that may be in suspension, through a small filter, this is dried and repeatedly extracted with boiling chloroform. The chloroform extract is filtered into the original indigo flask, the chloroform distilled off, the residue dried as before, and while still warm treated with 3 or 4 c.c. of concentrated sulphuric acid. The entire residue should be brought into solution by careful agitation. After standing for twenty-four hours the contents of the flask are poured into 100 c.c. of cold water; the flask is rinsed and the washings added to the solution. This is filtered once more and titrated with the permanganate solution. At first the blue color of the solution changes but little; later it turns greenish, and finally becomes yellowish or entirely colorless—not red. As a rule, the end-reaction is quite distinct, but the titration requires experience. The best results are obtained when from 10 to 15 c.c. of the dilute

permanganate solution are used. The resulting amount of indigo contained in the measured-off quantity of the first filtrate is then ascertained as described above.

Example.—Amount of urine: 1780 c.c.

The stock solution of potassium permanganate contains 3 grammes to the liter; 1 c.c. = 0.00596 gramme of oxalic acid = 0.0062 gramme of indigo. Diluted solution (5 : 200); 1 c.c. = 0.00015 gramme of indigo. 300 c.c. of urine were precipitated with 25 c.c. of the lead solution; 250 c.c. of the filtrate, corresponding to 230.7 c.c. of urine, treated with 250 c.c. of Obermayer's reagent. Extracted twice with chloroform. 4.3 c.c. of the permanganate solution were used in the titration = 0.00065 gramme of indigo, corresponding to 0.005 gramme in the 1780 c.c., according to the equation

$$230.7 : 0.00065 :: 1780 : x; \quad x = \frac{1.157}{230.7} = 0.005.$$

Other methods for the quantitative estimation of indican which have heretofore been used, with the exception of the spectroscopic method of Müller, are not only inaccurate, but, like this, too time-consuming and complicated to be of value to the practising physician. As a consequence almost all observers have based their conclusions upon an approximative estimation only. For practical purposes this is sufficient, and even Wang's method, though accurate and simple, will hardly find a ready entrance into the clinical laboratory, as it is still too time-consuming and too expensive for daily use. For scientific purposes, however, it may be recommended.

Urohæmatin.[1]—Urohæmatin appears to be the chromogen of the red pigment of the urine, and is very likely closely related to indoxyl. Little is known of its chemical composition or of its mode of formation. In all probability the red pigment which may be obtained from this substance is identical with other red pigments which have been described from time to time as occurring in the urine, such as that of Scherer, the urrhodin of Heller, the urorubin of Plosz, Schunk's indirubin, Bayer's indigo-purpurin, Giacosa's pigment, and also the indigo-red obtained by Rosenbach and Rosin by careful oxidation of the urine with nitric acid.

Further investigations are necessary before this subject can be fully understood; but bearing in mind the probable origin of urohæmatin from indoxyl, it would possibly be best to speak of the red pigment as indigo-red. In accordance with the view that urohæmatin is an indoxyl derivative, its clinical significance is similar to that of indican (which see).

The presence in normal urine of urohæmatin—*i. e.*, a chromogen yielding a red pigment when treated with certain reagents—may be demonstrated by shaking urine with chloroform and decanting after

[1] G. Harley, Verhandl. d. physik. med. Ges. z. Würzburg, 1855, vol. v. p. 1.

several days, when the addition of a drop of hydrochloric acid to the chloroform extract will cause the appearance of a beautiful rose color; this varies in intensity according to the amount of the chromogen present.

The purplish color so often obtained in the chloroform extract when Stokvis' modification of Jaffé's indican test is employed is due to a mixture of indigo-blue and indigo-red. Indican, however, is generally present in larger amounts than urohæmatin. In normal and, usually also, in pathological urines a red color is not obtained with the test mentioned. In a few isolated cases of ileus, peritonitis, and carcinoma of the stomach I have found more indigo-red than indigo-blue.

The so-called "Reaction of Rosenbach" is a convenient test for indigo-red when this is present in increased amounts: the boiling urine is treated drop by drop with concentrated nitric acid, when in the presence of large amounts of indigo-red it assumes a dark Burgundy color, which sometimes takes on a bluish tinge when held to the light. Owing to a precipitation of the pigment the mixture at the same time becomes cloudy and the foam assumes a blue color. In well-marked cases the Burgundy color does not appear to be changed by the further addition of nitric acid, but will sometimes suddenly change from red to yellow when 10–20 drops of the acid have been added. This reaction Rosenbach[1] regarded as symptomatic of various forms of severe intestinal disease associated with an impeded resorption throughout the entire intestinal tract. Ewald[2] likewise noted this reaction in cases of extensive disease of the small intestine, in carcinoma of the stomach, and in acute and chronic peritonitis; but he obtained negative results in carcinoma of the colon, stricture of the œsophagus, chronic diarrhœa, etc. *Rosenbach's reaction should be viewed in the same light as a highly increased elimination of indican.* I have met with the reaction in all conditions associated with greatly increased intestinal putrefaction, and, like Ewald, failed to note the reaction in a few cases of occlusion of the large intestine, in which an increased elimination of indican is likewise never observed.

Uroroseïnogen.[3]—In addition to indican and urohæmatin, still another chromogen, which yields a rose-red pigment when treated with mineral acids, appears to occur in normal urine, although in small amounts. Beyond the fact that the chromogen is not a conjugate sulphate, practically nothing is known of its chemical nature. The pigment, which has received the name *uroroseïn*, or Harnrosa, appears to be identical with Heller's urophaïn. Uroroseïn is best

[1] Rosenbach, Berlin. klin. Woch., 1889, vol. xxvi. pp. 5, 490, and 520, and 1890, vol. xxvii. p. 585.
[2] Ewald, Ibid., 1889, vol. xxvi. p. 953.
[3] H. Rosin, Deutsch. med. Woch., 1893, p. 51.

demonstrated by treating 5–10 c.c. of urine with an equal amount of concentrated hydrochloric acid, and 1 or 2 drops of a concentrated solution of sodium hypochlorite, when in the presence of much indican the mixture assumes a dark-greenish, blackish, or dark-blue color, owing to the formation of indigo. When the mixture is shaken with chloroform the supernatant fluid exhibits a beautiful rose color, which is due to the uroroseïn. This may now be extracted with amyl alcohol and separated from other pigments which are present at the same time, by shaking with sodium hydrate, whereby the solution is decolorized. Upon the addition of a drop or two of hydrochloric acid to the alcoholic extract the rose color reappears. Such solutions, however, soon become decolorized upon standing. A rose-red ring, referable to this pigment, is also frequently obtained in pathological urines when the ordinary nitric acid test is employed.

While normally uroroseïn is obtained only in traces, appreciable amounts are often met with in pathological conditions associated with grave disturbances of nutrition, as in nephritis, diabetes, carcinoma, dilatation of the stomach, pernicious anæmia, typhoid fever, phthisis, and at times in profound chlorosis, etc. A vegetable diet also appears to cause an increase in the amount of the chromogen.

Pathological Pigments and Chromogens.—The Blood-pigments.—The blood-pigments proper which may occur in the urine have already been considered (see page 490), and in this connection it will only be necessary to refer briefly to the occasional presence of hæmatin, urorubrohæmatin, urofuscohæmatin, and hæmatoporphyrin.

HÆMATIN is only rarely found. In order to demonstrate its presence, the urine is rendered strongly alkaline with ammonia, filtered, and the filtrate examined spectroscopically, when the spectrum shown in Fig. 6 will be noted; this may be changed into the spectrum represented in Fig. 7 by the addition of ammonium sulphide.

URORUBROHÆMATIN and UROFUSCOHÆMATIN were observed by Baumstark[1] in the urine of a case of pemphigus leprosus complicated with visceral lepra; they appear to be closely related to hæmatin. The color of the urine in this case varied between dark red and brownish red, strongly suggesting the presence of blood. In order to separate the pigments, the urine was dialyzed and the contents of the dialyzer dissolved in sodium hydrate solution. Upon the addition of hydrochloric acid to this solution a brown pigment separated out in flakes, while a second pigment remained in solution, imparting to it a beautiful red color. Upon filtration the acid filtrate was again subjected to dialysis, when the

[1] F. Baumstark, Pflüger's Archiv, 1874, vol. ix. p. 568. See, also, J. W. Schultz, Diss., Greifswald, 1874.

red pigment likewise separated out. The former substance Baumstark termed urorubrohæmatin, and the latter urofuscohæmatin.

Urohæmatoporphyrin has the formula $C_{16}H_{18}N_2O_3$, and is probably identical with the hæmatoporphyrin resulting from the action of sulphuric acid upon hæmatin. McMunn found a pigment answering the description of this substance in the urine in cases of rheumatism, Addison's disease, pericarditis, and paroxysmal hæmoglobinuria, which he termed urohæmatin, but which in all probability was hæmatoporphyrin. Le Nobel found the same pigment in two cases of hepatic cirrhosis and in one case of croupous pneumonia. Others have likewise met with hæmatoporphyrinuria in various forms of hepatic disease, as also in phthisis, exophthalmic goitre, typhoid fever, and hydroa æstivalis; further, in association with intestinal hemorrhages, in cases of lead poisoning, and especially during long-continued use of sulphonal, trional, and tetronal. Nebelthau records the history of a female patient, the subject of congenital syphilis, who had passed dark-red urine as long as she could remember, and continued to do so while under observation. Recent researches, moreover, have shown that in traces at least the substance is present in every urine. As regards the origin of these normal traces, the evidence is in favor of the view that they are formed within the body during its normal metabolism, and most likely in the liver, whence the substance is eliminated in the bile. A portion then escapes with the feces, while a similarly small amount is resorbed and eliminated in the urine. Increased amounts would accordingly suggest the existence of a hepatic insufficiency; and, as a matter of fact, we find that actual anatomical lesions then not infrequently occur. Taylor and Sailer thus report that in their case of sulphonal poisoning widespread degeneration of the hepatic cells existed; and Neubauer was able to isolate the pigment from the liver of rabbits to which sulphonal had been administered, while it was absent in all other organs. On the other hand, it is difficult to ascribe all the phenomena of such hæmatoporphyrinuria to hepatic changes, seeing that changes of like degree may occur without conspicuous urinary abnormality, and there is still much that is obscure in this condition.

Stokvis attributed the increased elimination of hæmatoporphyrin in cases of lead poisoning and following the continued use of sulphonal to the occurrence of hemorrhages into the intestinal mucosa, and suggested that the transformation of the hæmoglobin into hæmatoporphyrin was favored by the sulphonal. But while intestinal hemorrhages may occur in the sulphonal cases, they are not always observed, and, as Garrod points out, Kast and Weiss, as also Neubauer, were unable to verify the recorded experiments of Stokvis, in which he claims to have obtained a small amount of hæmatoporphyrin when fresh blood was digested with pepsin-hydrochloric acid and sulphonal at from 38° to 40° C.

Urines which contain much hæmatoporphyrin are usually dark red in color, but the shade may vary from a sherry or port-wine tint to a dark Bordeaux. It is noteworthy, however, that this color is not primarily due to the exaggerated degree of hæmatoporphyrinuria, but, as Hammarsten first pointed out, to other abnormal pigments which are but little known, but which are probably closely related to hæmatoporphyrin. As Garrod says, the removal of the hæmatoporphyrin from such urines causes little or no change of color, and when this pigment is added to normal urine until on spectroscopic examination bands of similar intensity are seen the change of tint produced is comparatively slight. In one such case, not due to sulphonal, he was able to isolate a purple pigment which differed in its properties from any known urinary coloring-matter, and to which the color of the urine in question was obviously in the main due. Neumeister also states that in sulphonal intoxication an iron-containing derivative of hæmoglobin occurs in the urine, which presents a reddish-violet color and shows a single band of absorption in the blue portion of the spectrum immediately bordering on the green.

Albumin is not present in uncomplicated cases of hæmatoporphyrinuria, and the pigment itself does not give the albumin reactions.

To test for hæmatoporphyrin, the following procedure may be employed:

Thirty c.c. of urine are treated with an alkaline solution of barium chloride. The precipitate, after having been washed with water and then with absolute alcohol, is extracted with ordinary alcohol acidulated with hydrochloric acid, by rubbing in a mortar. The solution thus obtained will present a reddish color in the presence of hæmatoporphyrin, and its filtrate yields the characteristic spectrum of the latter substance—*i. e.*, four bands of absorption, of which two are broad and dark and two light and narrow. The former alone are characteristic, and frequently the only ones visible. One of these extends beyond *D* into the red portion of the spectrum, while the other is situated between *b* and *F*. Of the other two bands, one may be seen between *C* and *D* and the other between *D* and *E*, nearer *E* (Fig. 10).

Garrod's Method.—To demonstrate the presence of hæmatoporphyrin under normal conditions, or when small amounts only are present in the urine, Garrod's method should be employed. To this end, several hundred c.c. of urine (500–1500) are treated with a 10 per cent. solution of sodium hydrate in the proportion of 20 c.c. of the alkali solution for 100 c.c. of urine. The precipitated phosphates are filtered off and thoroughly washed by repeatedly suspending them in water. Should the precipitate be of a reddish color, or if it shows the spectrum of hæmatoporphyrin in alkaline solution when examined on the filter in the moist state, we may con-

clude that much hæmatoporphyrin is present. In this case it is washed until the filtrate is colorless. If traces only are present, however, one washing must suffice. The precipitate is then treated with alcohol, which is acidified with hydrochloric acid to such an extent that the phosphates are entirely dissolved. The resulting solution should not exceed 15 to 20 c.c. in volume. This is then examined in a layer, of not less than 3 to 4 cm. in thickness, for the spectrum of acid hæmatoporphyrin, using a spectroscope with slight dispersion. The solution is now rendered alkaline with ammonia and treated with an amount of acetic acid which just suffices to redissolve the precipitated phosphates. On shaking with chloroform this extracts the pigment, and the chloroform solution then gives the spectrum of the alkaline hæmatoporphyrin, since organic acids do not change the pigment to the form which yields the acid spectrum. The residue which remains after evaporating the chloroform can finally be washed with water and dissolved in alcohol, when a nearly pure solution is obtained, which is comparable with a solution of hæmatoporphyrin obtained from hæmatin.

Precautions: If a preliminary test shows that the urine contains but little phosphates, a small quantity of calcium phosphate in acetic acid is added before the urine is rendered alkaline with the sodium hydrate solution. As hæmatin and chrysophanic acid are also precipitated with the phosphates, their absence must be insured. For this reason the urine should contain no rhubarb or senna.

In conclusion, it may be said that a chromogen of hæmatoporphyrin is also usually present in urines containing the free pigments, which probably explains why such urines gradually become darker on standing.

LITERATURE.—A complete account of the literature on hæmatoporphyrinuria up to 1893 is given by R. Zoja, "Su gualche pigmento di alcune urine," etc., Arch. ital. di clin. med., 1893, vol. xxxii. p. 63. A. E. Garrod, loc. cit.; and Centralbl. f. inn. Med., 1897, No. 21. Taylor and Sailer, Contributions from the William Pepper Laboratory, Philadelphia, 1900, p. 120. O. Neubauer, Arch. f. exper. Path. u. Pharmakol., 1900, vol. xliii. p. 455. B. J. Stokvis, "Zur Pathogenese d. Hæmatoporphyrinurie," Zeit. f. klin. Med., vol. xxviii. p. 1. Kast u. Weiss, Berlin. klin. Woch., 1896, vol. xxxiii. p. 621. Hammarsten, "Skandin. Arch. f. Physiol.," 1891, vol. iii. p. 31. Neumeister, Physiol. Chem., Jena, 1897. Nebelthau, Zeit. f. physiol. Chem., 1899, vol. xxvii. p. 324. B. Ogden, Boston Med. and Surg. Jour., 1898.

Biliary Pigments.—Of the four biliary pigments, viz., bilirubin, biliverdin, biliprasin, and bilifuscin, the former alone is met with in freshly voided urines, while the others may form upon standing, being oxidation-products of bilirubin. The pigment is never found in normal urine, and its occurrence may be regarded as a positive symptom of disease.

In health it will be remembered that BILIRUBIN, $C_{16}H_{18}N_2O_3$, formed in the liver from blood-pigment, is eliminated into the small intestine, in which it is transformed into hydrobilirubin and largely

excreted as such in the feces, while a small portion is reabsorbed into the blood and eliminated in the urine as urochrome or normal urobilin. Whenever, then, the outflow of bile into the intestines becomes impeded bilirubin is absorbed by the lymphatics and eliminated in the urine.

Among the numerous causes which give rise to *choluria* under such conditions may be mentioned obstruction of the biliary ducts, and especially of the common duct, referable to simple swelling of its mucous membrane, as in the ordinary forms of catarrhal jaundice. It may also be due to the presence of a biliary calculus, to parasites, compression of the duct by tumors of the liver, the gall-bladder, the duct itself, and of neighboring structures, and particularly of the pancreas, stomach, and omentum. Whenever the blood-pressure in the liver is lowered, so that the tension in the smaller biliary ducts becomes greater than that in the veins, choluria likewise results. The icterus occurring under all such conditions has been termed *hepatogenic icterus*, in contradistinction to the form observed in cases in which the liver has either totally or partially lost the power of forming bile, be this owing to the existence of degenerative processes affecting its glandular epithelium, as in cases of acute yellow atrophy, or to destruction of red corpuscles going on so rapidly and so extensively that the organ is incapable of transforming into bilirubin all the blood-pigment which is carried to it. This occurs in pernicious anæmia, malarial intoxication, typhoid fever, poisoning with arsenious hydride, etc. Icterus neonatorum is probably to a certain extent also dependent upon the latter cause. To this form the term *hæmatogenic icterus* has been applied. In such cases the occurrence of bilirubin in the urine can only be explained by assuming that a transformation of blood coloring-matter into bilirubin has taken place in the blood itself or in other tissues of the body. As a matter of fact, it appears to be generally accepted that such a transformation *can* actually occur outside of the liver, as the hæmatoidin which may be found in old extravasations of blood seems to be identical with bilirubin. On the other hand, however, the existence of a hæmatogenic icterus is positively denied, especially by Stadelmann. In accordance with his view, it may be demonstrated that in cases of pernicious anæmia, malaria, etc., the urine does not contain bilirubin, but usually urobilin. In cases of this kind which I had occasion to examine, bilirubin was never found. Further investigations are necessary to settle this question definitely.

Usually the presence of biliary pigment may be recognized by direct inspection, as urines which contain this in notable amounts present a color varying from a bright yellow to a greenish brown. Any morphological elements which may occur in the sediment are stained a golden yellow, and the same color is imparted to the foam of the urine as well as to the filter-paper used in the filtration. At

times, however, and particularly in cases in which the icterus is only beginning to appear, the presence of bilirubin is not infrequently overlooked, and urines containing urobilin in large amounts may be similarly mistaken for icteric urines. In doubtful cases, therefore, whether icterus exists or not, but in which the urine presents an intense yellow color, it is necessary to have recourse to chemical tests. A large number of these have been devised for the purpose of demonstrating the presence of bilirubin, all of which are fairly reliable. Only those will be described which I have examined myself and which are especially delicate.

Smith's Test.[1]—Five to 10 c.c. of urine are placed in a test-tube and treated with 2 or 3 c.c. of tincture of iodine (which has been diluted with alcohol in the proportion of 1 : 10) in such a manner that the iodine solution forms a layer above the urine. In the presence of bilirubin a distinct emerald-green ring is seen at the zone of contact. This test can be highly recommended, as it is exceedingly simple and not surpassed in delicacy by any other.

Huppert's Test.[2]—Ten to 20 c.c. of urine are precipitated with milk of lime (a solution of barium chloride is, perhaps, still more convenient), and the precipitate after filtering brought into a beaker by perforating the filter and washing its contents into the latter with a small amount of alcohol acidulated with sulphuric acid. The mixture is boiled, when in the presence of bilirubin the solution assumes a bright emerald-green color. Huppert's test is as delicate as is that of Smith, but is not so convenient for the needs of the practising physician.

Gmelin's Test (*as modified by Rosenbach*).[3]—The urine is filtered through thick Swedish filter-paper, when the latter is removed and a drop of concentrated nitric acid, which has been allowed to stand exposed to the air for a short time, is placed upon its inner surface. In the presence of bilirubin a prismatic play of colors will be seen to occur around the nitric acid spot.

Gmelin's Test.[4]—The urine is treated with nitric acid, which is carried to the bottom of the test-tube by means of a pipette, so as to form a layer beneath the urine, when a color-play, as already described (page 493), will take place at the line of contact between the two fluids; the green color is the most characteristic.

In this connection a few words may also be said of the occurrence in the urine of biliary acids and cholesterin.

Biliary Acids.—These may usually be found in the urine whenever bile-pigment is present, so that their clinical significance is essentially the same as that attaching to bilirubin. Their demonstration is,

[1] W. G. Smith, Dublin Med. Jour., 1876, p. 449.
[2] Huppert, Arch. d. Heilk., 1867, vol. viii. pp. 351 and 476.
[3] Rosenbach, Centralbl. f. d. med. Wiss., 1876, vol. xiv. p. 5.
[4] Tiedemann u. Gmelin, Die Verdauung nach Versuchen, Heidelberg, 1826, I. p. 80.

however, attended with such difficulties that the methods devised for this purpose may well be omitted at this place (see also page 228).

Cholesterin.—Cholesterin has never been found in icteric urines, and is only rarely seen in other pathological conditions. It has been observed in cases of chyluria, fatty degeneration of the kidneys, diabetes, in one case of epilepsy, in eclampsia, and in several cases of pregnancy. v. Jaksch has noted the presence of cholesterin crystals in a urinary sediment in a case of tabes and cystitis. Glinsky records a similar observation. Harley found it repeatedly in cases of pyuria. Reich states that he found cholesterin crystals of the size of a dollar in the urine of a case of chronic cystitis. Hirschlaff found larger quantities in the urine of a case of hydronephrosis; on one occasion 5.8 grammes in 100 c.c. of urine. I have found cholesterin crystals in the sediment in a case of acute nephritis. The urine was of a dark amber color, cloudy, of an acid reaction, and a specific gravity of 1.028. In the sediment numerous hyaline and epithelial casts and some red blood-corpuscles were found. Güterbock described a urinary calculus obtained from the bladder of a woman which consisted almost entirely of cholesterin (see also Feces). Langgaard noted the presence of the substance in a case of chyluria.[1]

Pathological Urobilin.—This pigment should not be confounded with the urochrome or normal urobilin described above, to which it is closely related, but from which it may be distinguished by means of the spectroscope. Gautier states that pathological urobilin may be obtained from urochrome by submitting the latter to the action of reducing agents; and, as I have already pointed out, Riva and Chiodera obtained a substance from urobilin by the action of potassium permanganate, which closely resembles urochrome. It is said to be identical with the *stercobilin* found in the feces, but differs from Maly's hydrobilirubin in containing a much smaller percentage of nitrogen, viz., 4.11, as compared with 9.22 (Garrod and Hopkins). While its occurrence in the urine is essentially a pathological phenomenon, it is at times also met with in normal urine, and appears to be derived from a special chromogen, *urobilinogen*, from which it may be set free by the addition of an acid. Both urobilin and its chromogen are precipitated by saturating the urine with ammonium sulphate, and both are soluble in chloroform. According to Maly, urobilin is formed by the reduction of bilirubin in the intestine, and is then in part resorbed and eliminated in the urine. Hayem, on the other hand, proposed the hypothesis that the substance originates in a diseased or disordered liver, as bilirubin does in the same organ in health, and accordingly he regards the appear-

[1] v. Jaksch. Klinische Diagnostik, 4th ed. p. 339. Glinsky, Maly's Jahresber., 1894, vol. xxiii. p. 484. Langgard, Virchow's Archiv, vol. lxxxvi. W. Hirschlaff, Deutsch. Arch., 1899, vol. lxii. p. 53.

ance of much urobilin in the urine as evidence of hepatic insufficiency. Others, again, maintain that urobilin is formed in the tissues at large either by the reduction of bilirubin or directly from the blood-pigment. The first view is notably held by Kunkel, Mya, Giarré, and others, while the hæmatogenous theory is notably represented by Gerhardt. Garrod discusses these various hypotheses at some length in his most interesting lecture on the urinary pigments in their pathological aspects, in which he personally inclines to the intestinal theory, as now held by Müller, Schmidt, Esser, and others. In a work of this scope it would lead too far to discuss the various investigations which lend themselves in support of this view, and I can here quote only the following from Garrod's paper: the chief seat of the formation of urobilin (for it is convenient to employ this term as including both pigment and chromogen) is undoubtedly the intestinal canal. This can only be gainsaid by denying the identity of the urinary and fecal pigments. The quantity normally present in the feces is far larger than that which enters the intestine with the bile (when a small amount is found), and there is strong evidence that the urobilin in bile is itself of intestinal origin. This being so, it is clear that theories other than the intestinal and its modifications merely attempt to trace a second source for the urobilin of the urine. It is equally clear that the substance from which the intestinal urobilin is formed is the bile-pigment. Under ordinary conditions the bile-pigment is destroyed in its passage along the intestine, and does not appear as such in the feces. In its place we find large quantities of urobilin, which in its turn disappears when occlusion of the common duct prevents the entrance of bile into the intestine. Again, when under certain morbid conditions the bile-pigment passes along the intestine unaltered, urobilin is absent from the feces. However, the conversion of bilirubin into urobilin is no mere process of reduction, but involves a much more radical change, with elimination of nitrogen. That the change is brought about by bacterial action there is much evidence to show. When bile is inoculated with fecal material and kept in an incubator a formation of urobilin rapidly takes place, and at the same time the bile-pigment diminishes, and ultimately disappears.

From its frequent occurrence in febrile urines pathological urobilin has also received the name *febrile* urobilin. It is, however, also observed in many other conditions, and especially in cases presenting the so-called hæmatogenic form of icterus, from which fact, indeed, and the usual absence of bilirubin at the same time, this form has been termed *urobilin icterus*.

Urobilinuria has further been observed in certain hepatic diseases. In twelve cases of atrophic and hypertrophic cirrhosis v. Jaksch was able to demonstrate the presence of urobilin in every instance,

a point which may at times be of considerable diagnostic importance, providing that other causes which are known to lead to urobilinuria can be eliminated. I have observed urobilin in a few cases of hepatic cirrhosis, chronic malaria, and pernicious anæmia, in all of which the skin presented a light icteric hue, and in which bile-pigment was absent from the urine. Unfortunately, an examination of the blood was not made, and I have hence not been able to confirm the statement of v. Jaksch that bilirubin occurs in the blood in almost every case in which urobilin is present in the urine. Urobilin has also been noted in cases of carcinoma, scurvy, Addison's disease, hæmophilia, in cases of retro-uterine hæmatocele, in extra-uterine pregnancy, following intracranial hemorrhages, etc. According to Bargellini, the degree of constipation in simple atony of the bowel is without influence upon the amount of urinary urobilin, but he states that in typhoid fever it causes an obvious increase; whereas disinfection or emptying of the large bowel produces a notable diminution in the amount.

Urines rich in urobilin usually present a dark-yellow color which is strongly suggestive of the presence of bilirubin; even the foam in such cases may be colored, making the resemblance between the two pigments still more complete. v. Jaksch points out, however, that urines containing indican in large amounts often likewise present a very dark-yellow color, a statement with which my own observations are in perfect accord. In every case a more detailed chemical examination should hence be made.

Gerhardt's Test.—If the urine contains much urobilin, which the color will indicate, 10–20 c.c. are extracted with chloroform by shaking, and the extract treated with a few drops of a dilute solution of iodo-potassic iodide. Upon the further addition of a dilute solution of sodium hydrate the chloroform extract is colored a yellow or yellowish-brown, and exhibits a beautiful green fluorescence; this is even more intense than that noted in the case of normal urobilin.

Spectroscopic Examination.—This is necessary when Gerhardt's test yields a doubtful result. The urine is then best examined as follows: 50 c.c. of urine are extracted in a separation funnel with amyl alcohol, which takes up both the pigment and its chromogen. After standing for several hours the urine is allowed to flow away, by opening the stopcock, when the alcoholic extract is decanted from above, and is treated with a concentrated alcoholic and ammoniacal solution of zinc chloride. In the presence of urobilin the liquid shows a beautiful fluorescence, and on spectroscopic examination a single band of absorption is seen between *b* and *F*. In acid solutions, on the other hand, a single band is likewise obtained between *b* and *F*, but this extends to the right beyond *F*, and is much darker. Should the urine contain much urobilin, its special extraction is not necessary. In such an event the acid urine shows the acid spectrum,

while the alkaline band is obtained after the addition of ammonia (see also Bang's Test).

LITERATURE.—A. E. Garrod, loc. cit. A. E. Garrod and F. G. Hopkins, "On Urobilin," Jour. of Physiol., 1898, vol. xxii. p. 451. Maly, Centralbl. f. d. med. Wiss., 1871, vol. ix. p. 849. Hayem, Gaz. hebdom., 1887, vol. xxiv. pp. 520 and 534; and Gaz. des Hôp., 1889, vol. lxii. p. 1314. Kunkel, Virchow's Archiv, 1880, vol. lxxix. p. 655. Mya, Arch. ital. di clin. med., 1891, vol. xxx. p. 101; and Lo Sperimentale, 1896, vol. l. p. 71. Giarré, Ibid., 1895, vol. xlix. p. 89, and 1896, vol. l. p. 81. F. Müller, Schlesische Gesellsch. f. vaterländ. Kultur, January, 1892. A. Schmidt, Verhandl. d. XIII. Congress. f. inn. Med., 1895, p. 320. Esser, Untersuchungen über d. Entstehungsweise d. Hydrobilirubins, etc., Diss., Bonn., 1896. Bargellini, Lo Sperimentale, 1892, vol. xlvi. p. 119. v. Jaksch, Zeit. f. Heilk., 1895, vol. xvi. p. 48. D. Gerhardt, Zeit. f. klin. Med., 1897, vol. xxxii. p. 313.

Melanin and Melanogen.—In cases of melanotic disease it has been repeatedly observed that the urine, which usually and probably always presents a normal yellow color when voided, gradually becomes darker upon exposure to the air, and finally turns black. This phenomenon indicates without a doubt that such urines contain a chromogen, *melanogen*, which, upon oxidation, yields the black pigment noted in these cases, viz., *melanin*. As yet, it has not been possible to isolate this substance in pure form, and it is, indeed, not definitely determined that the black color in such urines is referable to a single pigment. Such urines generally contain melanin and its chromogen in solution; deposits of melanin granules by themselves are only occasionally seen, and are not characteristic, as they may also be found in cases of chronic malarial intoxication, etc., when they may, indeed, be met with in the blood, constituting the condition spoken of as *melanæmia*.

While the occurrence of melanin in the urine is probably indicative in most cases of the existence of melanotic tumors, it should be stated that this symptom cannot be regarded as pathognomonic, as it may be absent in the case of melanotic tumors, and present in wasting diseases and inflammatory affections, and may at times, though very rarely, even be associated with the presence of non-pigmented growths. Nevertheless, its occurrence should always be regarded with suspicion, and, taken in conjunction with other symptoms, will often lead to a correct diagnosis.

TESTS FOR MELANIN AND MELANOGEN.—1. The presence of melanogen may be assumed if upon the addition of ferric chloride solution a black precipitate appears in the urine, which is soluble in a solution of sodium carbonate and can then be reprecipitated as a black or brownish-black powder by means of mineral acids. Instead of the ferric chloride barium hydrate may also be used.

2. A few cubic centimeters of urine are treated with bromine-water when in the presence of melanin or melanogen a precipitate is obtained, which is yellow at first, but gradually turns black.

LITERATURE.—T. H. Eiselt, "Die Diagnose d. Pigmentkrebses durch d. Harn," Prag. Vierteljahrschr. f. praktische Heilk., 1858, iii. p. 190, and 1862, vol. iv. p. 26. Senator, "Ueber schwarzen Urin," Charité Annal., 1891. Hoppe-Seyler, Zeit. f. physiol. Chem., 1891, vol. xv. p. 179. F. Grohe, "Zur Gesch. d. Melanaemie," Virchow's Archiv, 1861, vol. xx. p. 306.

Phenol Urines.—The development of a dark-brown or black color upon standing is not always due to the presence of melanin, as a similar appearance may be noted in cases of poisoning with carbolic acid, following the ingestion of salol, hydrochinon, pyrocatechin, salicylic acid, etc., in large amounts. The color in such cases is due in all probability to the presence of various oxidation-products of hydrochinon, and in the last instance possibly to the so-called humin-substances.

The test referred to above will prevent confusion as to the origin of the color as far as melanin is concerned, and with the history of the case given, moreover, further chemical examination is generally unnecessary. In suspected cases of carbolic acid poisoning, however, the mineral as well as the conjugate sulphates should be quantitatively determined, when the factor $\frac{A}{B}$ (see Sulphates) will be found greatly diminished. If at the same time other factors, which might cause a greatly increased elimination of conjugate sulphates, can be excluded, the diagnosis of poisoning with carbolic acid or one of its derivatives may be inferred. Salol and salicylic acid may be recognized from the fact that such urines when treated with a solution of ferric chloride develop a marked violet color which does not disappear on standing. The reaction thus differs from that obtained with diacetic acid (see also page 573).

Alkapton.—Urines are at times, though very rarely, seen which, like the phenol urines, turn dark on standing, but in which the change in color is neither referable to the presence of phenol or its derivatives, nor to melanin. Such urines are of a normal color when passed, but gradually turn reddish brown upon exposure to the air. Treated with a small amount of alkali, this change occurs almost immediately. Fehling's solution is reduced on the application of heat, while bismuth is not affected. Ammoniacal silver solution is reduced in the cold, and a temporary bluish-green color develops when the urine is treated with a ferric salt. The fermentation test is negative, and examination with the polarimeter shows that the substance in question is not glucose. With phenylhydrazin no osazon is formed.

Bödeker, who first observed a urine of this kind, termed the substance giving rise to the reactions just described alkapton, and subsequently expressed the belief that his alkapton might possibly have been pyrocatechin. Subsequent investigators succeeded in isolating substances from such urines which have been variously termed pyrocatechuic acid, urrhodinic acid, glucosuric acid, uroleucinic acid, and uroxanthinic acid. Baumann and Wolkow later were able to isolate *homogentisinic acid* in pure form from the urine of such cases, and expressed the belief that some of the substances obtained by previous observers were in reality the same. Since that time this

acid has also been found by Garrod, Ogden, Stange, Stier, and others. There is reason to believe, however, that the reaction is not always due to one and the same substance.

Of the origin of alkapton little is known. Baumann expressed the opinion that homogentisinic acid might be derived from tyrosin, and that the condition is referable to the activity of special micro-organisms in the upper portions of the intestines. As a matter of fact the amount of homogentisinic acid can be very materially increased by the administration of tyrosin, and Mittelbach has shown that if the substance is given in frequently repeated and small doses almost the entire amount reappears in the urine as homogentisinic acid. Tyrosin, however belongs to the *para*-series, while homogentisinic acid is an *ortho*-compound, so that the transformation of tyrosin into homogentisinic acid cannot be a direct process, and it has accordingly been questioned whether Bauman's view regarding the origin of alkapton is correct. There is evidence indeed to show that homogentisinic acid does not originate in the intestines, viz., is not a product of bacterial activity. It has thus been found that the alkaptonuria does not cease during starvation, and that a restriction of the putrefactive processes in the intestines by means of oil of turpentine, a kefir diet, and the administration of β-naphthol does not lead to a diminished elimination of homogentisinic acid. It has never been found in the feces, moreover; and Garrod has shown that after inoculation of common bouillon, meat-juice, or tyrosin broth with alkaptonuric feces homogentisinic acid is not formed. Embden observed that when an alkaptonuric individual took homogentisinic acid by the mouth a far larger portion appeared in the urine than when the same substance was administered to a healthy individual, which suggests that the alkaptonuria may be referable to impairment of the normal processes of oxidation.

The prevailing view at the present time is accordingly that alkaptonuria is a metabolic anomaly comparable to glucosuria and cystinuria; but, unlike glucosuria, it can scarcely be regarded as an expression of a pathological process. It may, of course, occur in individuals, suffering from disease, and has thus been observed in connection with glucosuria, in acute gastro-intestinal catarrh, in phthisis, acute miliary tuberculosis, in one case of brain tumor, carcinoma of the prostate, etc. More frequently the condition is accidentally discovered in apparently healthy individuals, and has repeatedly been confounded with glucosuria owing to the positive reduction test with Fehling's solution.

Garrod, from an analysis of all the reported cases, concludes that the condition is nearly always congenital. In 32 known instances which were presumably congenital, 19 occurred in seven families. One family contained 4 alkaptonurics, three others 3, and the remaining three 2 each. In fully 60 per cent. of the cases, it appears

from Garrod's studies, the parents of alkaptonurics were first cousins. There is thus far only one known instance in which the anomaly has been transmitted by an alkaptonuric father to his son.

The condition commonly persists through years and perhaps a lifetime. It may also occur as a transitory abnormality, however, as is apparent from the case of Hirsch, in which the condition persisted for three days, and the case of Geyger, in which the alkaptonuria was observed on only two days. A few observers further report on the occurrence of alkaptonuria shortly preceding death.

The amount of homogentisinic acid eliminated in the twenty-four hours is variable, but usually large. Baumann found an average elimination of 4.6 grammes; the largest amount eliminated in twenty-four hours was 6 grammes. In Meyer's case, a child one and one-half years old, 3.3 grammes were passed pro die. Larger quantities are obtained after a liberal diet of meats than with a vegetable diet. By the administration of tyrosin the amount can be artificially increased; in one of Baumann's cases an elimination of 14 grammes in the twenty-four hours could be thus produced. Phenylpropionic acid and benzoic acid do not cause an increased excretion of homogentisinic acid. After the administration of phenylacetic acid, on the other hand, the power of reduction of the urine is at times increased, and following the ingestion of 10 grammes of phenylamidoacetic acid Embden noticed an increase in the power of rotation of 36.5 per cent. This, of course, suggests that the acid in question may be concerned in the production of increased amounts of homogentisinic acid, but actual transformation has not as yet been observed. A notable increase is also produced by phenylalanin.

To isolate homogentisinic acid from alkapton urines, and to determine its amount, BAUMANN'S METHOD may be employed. The collected urine of twenty-four hours is acidified with 250 c.c. of a 12 per cent. solution of sulphuric acid and extracted three times with an equal volume of ether. The ethereal extract is evaporated to a syrup. The crystals which separate out on standing are dissolved in 250 c.c. of water. This solution is brought near the boiling-point, and is then treated with 30 c.c. of a neutral lead acetate solution (1 : 5) and rapidly filtered. In the filtrate the lead salt crystallizes out in transparent needles and prisms. This is then decomposed with hydrogen sulphide and the filtrate carefully evaporated on a water-bath until the fluid begins to darken, when it is further concentrated in the vacuum to the point of crystallization. The resulting prismatic crystals are almost colorless and transparent. They melt at a temperature of 146.5°–147° C., and are readily soluble in water, alcohol, and ether, and are almost insoluble in chloroform, benzol, and toluol. A solution of the acid, which may thus be isolated in pure form, presents the same characteristics as the urine from which it was obtained.

The following method, suggested by Garrod, may also be employed, and has the advantage of greater simplicity.

Garrod's Method.—The urine itself is heated nearly to boiling without any preliminary treatment, and for each 100 c.c. of urine at least 5 or 6 grammes of solid neutral lead acetate are added.

As soon as the acetate is dissolved, the bulky gray precipitate which forms is removed by filtration, and the filtrate, which has a pale-yellow color, is allowed to stand for twenty-four hours in a cool place. If the urine be very rich in homogentisinic acid, or if the flask containing it be placed upon ice, minute acicular crystals, which are almost colorless, quickly form; but as a rule crystallization does not commence until several hours have elapsed. The crystals are then much larger, are grouped in stars or rosettes, and are more deeply colored.

In summer weather it would probably be desirable to start the crystallization by artificial cooling; but although the process is greatly accelerated at a low temperature, the total yield is not materially increased.

If formation of the crystals be long delayed, the liquid may be warmed again and more lead acetate added.

After the lapse of twenty-four hours crystals cease to form, even when the liquid is placed upon ice.

The crystalline product so obtained is lead homogentisinate. When the crystals are dissolved in hot water the solution assumes a deep-brown color with alkalies; it reduces Fehling's solution readily with the aid of heat, and yields a transitory deep-blue color with a dilute solution of ferric chloride. From the lead salt free homogentisinic acid may be obtained by decomposing it with hydrogen sulphide.

Estimation.—Entirely satisfactory methods for the quantitative estimation of homogentisinic acid in the urine are not available. The following method, however, is sufficiently accurate for clinical purposes: 50 c.c. of urine are treated with 15 grammes of ammonium chloride, which should be brought into solution by shaking, in a stoppered graduate. After standing for about twelve hours to allow the uric acid to separate out the solution is filtered and an accurately measured portion of the filtrate titrated with a decinormal ammoniacal solution of silver nitrate. The titration is continued until a further reduction of the silver solution does not occur, which is ascertained by acidifying a few drops of the filtered mixture with hydrochloric acid, when in the presence of free silver a turbidity referable to silver chloride occurs. Accuracy within narrower limits than $\frac{1}{4}$ c.c. is scarcely possible, as the turbidity referable to silver chloride can only be recognized within 0.2–0.3 c.c. According to Baumann, 240–245 c.c. of the silver solution represent 1 gramme of homogentisinic acid.

PLATE XX.

Ehrlich's Diazo-Reaction, as modified by the author. The orange color in the lower portion of the test tube may be obtained in any urine; the dark carmine ring indicates the presence of the reaction in a well-pronounced degree; the colorless zone above is intended to indicate the ammonia that has been added.

LITERATURE.—Bödeker, Annal. d. Chemie u. Pharmakol., 1861, vol. cxvii. p. 98. Baumann u. Wolkow, Zeit. f. physiol. Chem., 1891, vol. xv. p. 228. Stier, Berlin. klin. Woch., 1898, vol. xxxv. p. 185. Embden, Zeit. f. physiol. Chem., 1893, vol. xvii. p. 182, and vol. xviii. p. 304. Ogden, Zeit. f. physiol. Chem., 1895, vol. xx. p. 280. Futcher, N, Y. Med. Jour., 1898, vol. lxvii. p. 69. Garrod, Jour. Physiol., 1899, vol. xxiii. p. 512; and Med.-Chir. Trans. Royal Soc., vol. lxxxii. p, 367. E. Meyer, Deutsch. Arch., vol. lxx. Hefte 5 u. 6. F. Wittelbach, Ibid., 1901, vol. lxxi. p. 50.

Blue Urines.—Blue urines are sometimes seen, the color of which is due to indigo formed from urinary indican, in all probability within the urinary passages. Their occurrence can only be regarded as a medical curiosity. One case of this kind is reported by McPhedran and Goldie,[1] in which after direct extraction of the urine with ether only a faint reaction was obtained on further examination, and which probably was referable to incomplete previous extraction. Formerly, when indigo was employed in the treatment of epilepsy, blue urines were frequently seen. At the present time, when methylene-blue is occasionally used in the treatment of malaria and chyluria, this pigment is found in the urine.

Green Urines.—Green urines have also been described; the cause of the color, however, has not been definitely ascertained.

Pigments referable to Drugs.—Certain drugs may also cause changes in the normal color of urine, and in doubtful cases inquiry in this direction should be made. It has been pointed out that carbolic acid, hydrochinon, pyrocatechin, and salol cause the appearance of a dark-brown color, and that after the administration of indigo and methylene-blue blue urines are voided. Santonin, rheum, and senna color urines a bright yellow, so that they may resemble icteric urines in appearance. The yellow color in such cases is changed to an intense red by the addition of an alkali, and, if ammoniacal fermentation is going on at the same time in the bladder, the patient may believe himself to be suffering from hæmaturia. The red color thus produced is due to the action of the alkali upon chrysophanic acid. When urines containing copaiba are treated with hydrochloric acid a red color results, which changes to violet upon the application of heat. During the administration of potassium iodide, or the use of iodine in any form, a dark mahogany color is obtained when the urine is treated with nitric acid. In doubtful cases Stokvis' modification of Jaffé's test for indican should be employed, when in the presence of an iodide the chloroform assumes a beautiful rose-red color.

For the detection of other drugs and poisons in the urine the reader is referred to special works.

Ehrlich's Reaction.—Under certain pathological conditions, and especially in typhoid fever, a chromogen may be present in the urine, which, when treated with diazo-benzene-sulphonic acid and ammonia, imparts a distinct red color to the urine, varying from eosin to a deep garnet-red (Plate XX.). This reaction, which is generally

[1] Trans. Assoc. Am. Phys., 1901.

spoken of as Erhlich's reaction, or the *diazo-reaction*, was at one time regarded as pathognomonic of typhoid fever. Subsequent examinations, however, have shown that it may also be present in other diseases. Michaelis, who has made an exhaustive study of this question, divides into four groups the diseases in which the reaction has been observed. In the first group, comprising diseases of the nervous system, chronic diseases of the heart and kidneys, malignant tumors, etc., the reaction is rarely seen. When present, it usually indicates a secondary infection. The second group includes those diseases in which the reaction is almost always present, namely, typhoid fever and measles. In the diseases of the third group it is often, though not invariably, observed. Under this heading are classed scarlet fever, erysipelas, pneumonia, diphtheria, pyæmia, acute miliary tuberculosis, etc. The fourth group comprises pulmonary tuberculosis, and includes acute caseous pneumonia.

The value of Ehrlich's reaction in typhoid fever was at first overestimated, but is at present certainly underestimated. I have personally studied this problem with great care, and after many years' experience maintain, as I did years ago, that the test is a most important diagnostic aid in the disease in question. As a general rule the reaction is present as early as the fifth or sixth day, and may persist into the third week; it then disappears, but may reappear when a relapse occurs. Excepting in children, its absence from the fifth to the ninth day usually indicates a mild case. This rule, however, is not without exception, and I have seen a case of typhoid fever in which notwithstanding exceedingly high temperatures (106.5° at 6 A. M.) the reaction was not obtained until the beginning of the third week, and then persisted for only a few days. When the reaction is continuously present after the third week I am inclined to suspect acute tuberculosis. It may be present as early as the fourth day of the disease.

In paratyphoid as in typhoid fever the reaction is also fairly constant.

Of late much attention has been paid to the occurrence of Ehrlich's reaction in pulmonary phthisis. As a result of his investigations Michaelis concludes that its presence in such cases indicates either that the process is very extensive or that it will progress very rapidly, and that the prognosis is grave. A cure, he believes, is impossible, and improvement, if any, only temporary. Clemens notes that of 100 cases of phthisis which ended fatally 87 showed the diazo reaction; Rütimeyer obtained positive results in 85 cases out of 106 which died. Of 13 cases of acute tubercular pneumonia Fränkel and Troje found a positive reaction in 11. Grundriss states that in his fatal cases the reaction was present without exception. Similar results have been obtained by Cnopf, Sée, Goldschmidt, and others. Michaelis himself reports

that of 111 cases of phthisis which were admitted to the Berlin Charité with well-marked reaction 80 died in the hospital, 13 were discharged unimproved, 3 were transferred to other hospitals, and 15 left improved. In other words, of these 111 cases a fatal result was known to have occurred in 72 per cent. Stadelmann states that of 38 other cases with positive reaction 28 died in the hospital —*i. e.*, about 75 per cent. The subsequent fate of the remaining cases was not ascertained; but we may well assume that of these at least 50 per cent. died, so that we may formulate the general rule that a fatal result may be anticipated in about 85 per cent. of all cases of phthisis in which a positive reaction is obtained. Michaelis, moreover, suggests that the end may be expected to occur within six months from the time at which a persistent Ehrlich's reaction is established. Exceptions occur, but the above is the rule. In Koch's institute at Berlin patients presenting the diazo reaction are not treated with tuberculin, as such cases are generally regarded as hopeless (Brieger).[1]

In tubercular peritonitis the diazo reaction is found in about 25 per cent. of all cases.

As regards the frequency of occurrence of the reaction in diphtheria, it appears from the observations of Rivier[2] and others that it is decidedly uncommon. Of his own 118 cases, and 44 additional onces collected from the literature, only 10 gave a positive result; and of these, 4 should be eliminated as they occurred in complicated cases, so that the reaction was absent in about 97 per cent.

In the scarlatiniform erythema due to serum treatment the reaction is apparently absent, while in true scarlatina it is fairly common. Including a number of cases collected from the literature Rivier found a positive reaction in 41 cases out of 73. He concludes that in the differential diagnosis between the two conditions scarlatina may be affirmed, if the reaction is positive, while if negative there is strong presumptive evidence against the disease.

In measles a positive reaction was obtained in 75 of 85 cases.

The reaction is possibly due to the presence of alloxyproteinic acid.[3]

As the preparation of chemically pure, crystalline diazo-compounds is a difficult process, Ehrlich uses sulphanilic acid, which, when treated with nitrous acid in a nascent state, gives rise to the formation of diazo-benzene-sulqhonic acid, as is shown by the equations:

[1] Discussion on Tuberculosis, Michaelis, Deutsch. med. Woch., 1901. v. b. p. 211.
[2] Rivier, Thèse de Paris, 1898.
[3] Bondzynski u. Panek, Berlin. d. deutsch. chem. Ges., 1903, vol. xxxv. p. 2951.

(1) $NaNO_2 + HCl = NaCl + HNO_2$.

(2) $C_6H_4(NH_2)(SO_3H) + HNO_2 = C_6H_4(N{=}N)(SO_3) + 2H_2O$.

Para-amido-benzene-sulphonic acid. Diazo-benzene-sulphonic acid.

This is the active principle in the mixture employed.

Other compounds may, of course, also be used, such as meta-amido-benzene-sulphonic acid, ortho- and para-toluidin-sulphonic acid, etc.; but of all these, Ehrlich found the common sulphanilic acid the most convenient. Two solutions, which must be kept in separate bottles, are employed. The one is a 5 per cent. solution of hydrochloric acid, to which sulphanilic acid is added in the proportion of 1 gramme for each 100 c.c. The other is a 0.5 per cent. solution of sodium nitrite.

The two solutions are mixed in the proportion of 40 to 1 immediately before using. A few cubic centimeters of urine are then treated with an equal volume of the reagent, the mixture is shaken and rendered alkaline with ammonium hydrate. This is best allowed to flow down the sides of the tube, so as to form a layer above the mixture. At the junction of the two fluids a colored ring will now be observed. With urines which do not contain the chromogen this will be a more or less distinct orange, while in its presence a red color is obtained. The intensity of this color may vary from eosin to a deep garnet-red. If the mixture is now agitated and the reaction is positive, the foam will likewise be colored red, and upon pouring the solution into a porcelain basin containing much water a beautiful salmon color is obtained, even if only traces of the chromogen are present. Carried out in this manner no question will arise as to the presence or absence of the reaction. Ehrlich states that on standing a green sediment forms in the alkalinized mixture, and he regards this sediment as especially characteristic. My experience has been that this becomes manifest only when the color-reaction is well pronounced, and I am inclined to attach more importance to the salmon color obtained upon copious dilution. With normal urines this is never obtained, and it can still be seen when inspection of the fluid in the test-tube would leave in doubt.

The older method of Ehrlich I have abandoned, as the test just described is simpler, and, in my experience, just as reliable. He advised the addition of about 50 c.c. of absolute alcohol to 10 c.c. of urine, subsequent filtration, and examination of the filtrate, as just described.

Greene states that if 1 part of the sodium nitrite solution is added to 100 instead of 40 parts of the sulphanilic acid solution, a positive reaction is no longer obtained in cases of croupous pneumonia and of pulmonary tuberculosis, while in typhoid fever the

Cases Tested.	Total Number.	Reaction.	
		Present.	Absent.
Typhoid fever	64	61[1]	3
Malarial fever	4	0	4
Tetanus	2	0	2
Acute miliary tuberculosis	3	0	3
Joint tuberculosis	4	0	4
Pulmonary tuberculosis	16	2	14
Septicæmia	4	3	1
Ulcerative endocarditis	1	0	1
Secondary syphilis	4	0	4
Erysipelas	2	0	2
Scarlatina	3	0	3
Measles	2	0	2
Carcinoma	4	2	2
Pneumonia	11	1	10
Rheumatism, chronic	10	0	10
Rheumatism, acute	5	0	5
Diphtheria	3	0	3
Diarrhœa	4	0	4
Appendicitis	3	0	3
Albuminuria of pregnancy	6	0	6
Chronic nephritis	19	0	19
Cystitis	2	0	2
Urethritis, specific	7	0	7
Oxaluria and lithæmia	11	0	11
Pleurisy	5	0	5
Pyæmic abscess of lung	1	0	1
Tuberculosis of prostate	3	0	3
Necrosis of long bones	2	0	2
Rötheln	1	0	1
Syphilis (third stage)	5	0	5
Alcoholic neuritis	3	0	3
Hysteria	6	0	6
Epilepsy	2	0	2
Leg ulcer, varicose	7	0	7
Fractures, long bones	5	0	5
Fracture, skull	2	0	2
Burns, severe	2	0	2
Gunshot wounds, aseptic	2	0	2
Morphin poisoning	1	0	1
Sciatica	3	0	3
Cirrhosis, hepatic	2	0	2
Simple enteritis	3	0	3
Angioneurotic œdema	2	0	2
Endometritis	3	0	3
Pericarditis	1	0	1
Meningitis	1	0	1
Vulvitis and vaginitis, specific	2	0	2
Orchitis, gonorrhœal	1	0	1
Valvular heart-disease	7	0	7
Quinsy and tonsillitis	3	0	3
Normal urines	30	0	30
Varicella	1	0	1
Typhoid relapse	3	3	0
Gastric ulcer	2	0	2
Acute bronchitis	3	0	3
Chronic constipation	7	0	7
	315		

[1] 95 per cent.

reaction occurs with the same intensity. It is thus possible that the test may be still further modified, and become even more valuable. On page 563 are given some of the results which Greene obtained with this method.

While in the absence of the chromogen, as I have already stated, a more or less pronounced orange color is usually obtained, exceptions have been noted. Ehrlich thus records that in urines containing biliary coloring-matter an intensely dark, cloudy discoloration occurs at times, which upon boiling is changed to a well-marked reddish violet. In rare instances of ulcerative endocarditis, hepatic abscess, and intermittent fever, Ehrlich further observed an intense yolk-yellow color, which was even imparted to the foam.

Of interest is the observation of Burghart, that after the administration of tannic acid, gallic acid, and certain iodine preparations, Ehrlich's reaction disappears from the urine. But, as Burghart himself suggests, it is likely that this inhibitory effect is not exerted upon the diazo-forming substance, but upon the reagents employed. Other factors, which may prevent the occurrence of Ehrlich's reaction, in pulmonary tuberculosis at least, are the occurrence of renal complications (albuminuria). Naphthalin, after its administration by the mouth, according to my experience causes a reaction, the color of which corresponds exactly to that of the diazo reaction.

LITERATURE.—Ehrlich, Zeit. f. klin. Med., 1882, vol. v. p. 285; Charité Annal., 1883, vol. viii. p. 28, and 1886, vol. xi. p. 139. Goldschmidt, Münch. med. Woch., 1886, vol. xxxiii. p. 35. Rütimeyer, Corresp. Blatt. f. Schweizer Aerzte, 1890, vol. xxvi. Greene, Med. Record, Nov. 14, 1896. C. E. Simon, Johns Hopkins' Hosp. Bull., 1890. J. Friedenwald, N. Y. Med. Jour., 1893. M. Michaelis, Berlin. klin. Woch., 1900, p. 274; and Deutsch. med. Woch., 1899, p. 156. J. R. Arneill, Am. Jour. Med. Sci., 1900, p. 296.

Ehrlich's Dimethylamidobenzaldehyde Reaction.—Ehrlich has shown that under various pathological conditions a fine cherry-red color develops on shaking a specimen of urine with a few drops of dimethylamidobenzaldehyde in acid solution, and that the resulting pigment can be in part extracted with chloroform, and almost entirely so with epi- or dichlorhydrin. With normal urines a similar reaction can be obtained, but it is much less intense, and if done at ordinary temperatures a distinct red color does not develop. On heating, however, it appears, and can likewise be extracted with epichlorhydrin. Of the nature of the substance which gives rise to the red color nothing definite is known.

The occurrence of the reaction in disease has been studied by Clemens, Kozickowsky, and myself. I can summarize my own results as follows: 1. A direct reaction, of pathological grade, does not occur in health. 2. A positive reaction is most commonly obtained in cases of tuberculosis. 3. It may also be seen in non-tubercular cases, both febrile and non-febrile. 4. It is not dependent upon the presence of the body which gives rise to the diazo

reaction. 5. For its production elevation of temperature, gastro-intestinal disturbances, and cyanosis are not essential. 6. Common to all cases seems to be an increased katabolism of the tissue albumins.

My positive results include cases of pulmonary tuberculosis, tuberculosis of the hip-joint, pneumonia, typhoid fever, appendicitis, embarras gastrique, icterus, malignant endocarditis, empyema, œsophageal carcinoma, and a remarkable instance of traumatic neurosis, in which a loss of weight of from sixty to seventy-five pounds had occurred.

My list of negative cases, on the other hand, includes, first of all, a large number of normal or supposedly normal individuals; in addition, cases of normal labor, neurasthenia, hysteria, diabetes, aortic aneurism, myelogenous leukæmia, lymphatic leukæmia, acute nephritis (scarlatinal), simple diarrhœa, morphinism, valvular disease, phthisis (stationary), diphtheria (before and after the use of antitoxin), typhoid fever, cases of abortion, appendicitis, influenza, chronic nephritis, cystitis, pyelitis (calculous), measles, tuberculosis of the hip-joint, cystic kidney, carcinoma of the kidney, tonsillitis, acute and chronic bronchitis, pneumonia, icterus, tuberculous peritonitis, general erythema, varicocele, following various operations, such as nephrorrhaphy, removal of pus tubes, operations for vesico-vaginal fistula, fistula in ano, and suspension of uterus. Examination of a urine containing cystin and diamins was also negative. A comparison of the negative with the positive cases will show at once that not all cases of pulmonary tuberculosis, tuberculous hip-joint disease, pneumonia, typhoid fever, appendicitis, and icterus give a positive result. So far as tuberculosis is concerned, however, it appears that the reaction is more likely to occur in the actively progressive cases than in those which are more or less stationary. It was also noted that the positive cases almost all gave a positive diazo reaction, while in the negative cases this was not obtained. Exceptions, however, may also occur.

In my personal examinations I employed a 2 per cent. solution of dimethylparamidobenzaldehyde in equal parts of water and concentrated hydrochloric acid. A few cubic centimeters of urine in a test-tube are treated with from 5 to 10 drops of the reagent; the mixture is set aside or agitated for a few minutes and the color then noted. Normal urines usually turn a greenish yellow, or the normal color merely becomes intensified. At times a dark amber color develops, though this is less common in health, unless the urine is brought to the boil before the reagent is added. In this way it is a common experience to meet with moderate or dark amber tints. With these reactions, however, I have not occupied myself, and, like Clemens and Koziczkowsky, I have only noted the reaction as positive when a distinct *cherry-red* color developed,

either immediately on adding the reagent or after agitation or standing.

LITERATURE.—Ehrlich, Med. Woch., 1901, No. 15, Clemens, Deutsch. Arch., 1901, vol. lxxi. p. 168. Koziczkowsky, Berl. med. Woch., 1902, vol. xxxix. No. 44. Simon, Am. Jour, Med. Sci., 1903, vol. cxxvi. p. 471.

CONJUGATE SULPHATES.

In addition to indoxyl (see Indican), skatoxyl, phenol, paracresol, and pyrocatechin occur in the urine in combination with sulphuric acid.

Skatoxyl.—Skatoxyl results from the skatol formed during the process of intestinal putrefaction, as indoxyl is derived from indol, and is partly eliminated in the urine as skatoxyl sulphate. Clinically it is of little interest, as the amount excreted is very small, and it is not necessary to enter into a further consideration of its chemical properties or mode of detection at this place (see Feces).

Phenol.—Phenol, according to Brieger, occurs only in very small amounts in human urine, the usual phenol reactions being largely referable to paracresol. Normally, about 0.03 gramme is eliminated in the twenty-four hours, but in pathological conditions much larger quantities may be found. Remembering the origin of phenol, it is clear that an increased elimination may be observed whenever putrefactive processes are going on in the tissues and cavities of the body, or whenever there is an increase in the degree of intestinal putrefaction, though in the latter condition the indican is usually the only conjugate sulphate that is found increased. In peritonitis, diphtheria, erysipelas, scarlatina, empyema, pulmonary gangrene, putrid bronchitis, etc., an increased elimination of phenol is commonly seen. Important from a diagnostic standpoint, further, is the fact that in uncomplicated cases of typhoid fever no increase is observed, while this is common in tubercular meningitis.[1] The largest amounts, of course, are seen in cases of poisoning with carbolic acid or one of its derivatives.

As the quantitative estimation of phenol is too complicated for the purposes of the general practitioner, Salkowski's qualitative test is here also described. From the intensity of the reaction certain conclusions may be drawn as to the amount present. It is especially serviceable in cases of suspected poisoning with carbolic acid.

Salkowski's Test.—About 10 c.c. of urine are boiled in a test-tube with a few cubic centimeters of nitric acid, and, on cooling, treated with bromine-water. The development of a pronounced turbidity or the occurrence of a precipitate indicates the presence of an increased amount of phenol.

[1] A. Strasser, "Ueber d. Phenolausscheidung bei Krankheiten," Zeit. f, klin. Med., vol. xxiv. p. 543. Brieger, Zeit. f. klin. Med., 1881, vol. iii. p. 468. Kast u. Boas, Münch. med. Woch., 1888, vol. xxxv. p. 55.

Quantitative Estimation.—*Principle.*—When potassium-phenyl sulphate is treated with hydrochloric acid, phenyl sulphate results, which further takes up one molecule of water, giving rise to the formation of sulphuric acid and phenol, according to the following equations:

$$(1)\quad SO_2\begin{matrix}O.C_6H_5\\OK\end{matrix} + HCl = KCl + SO_2\begin{matrix}O.C_6H_5\\OH\end{matrix}$$

$$(2)\quad SO_2\begin{matrix}O.C_6H_5\\OH\end{matrix} + H_2O = SO_2\begin{matrix}OH\\OH\end{matrix} + C_6H_5.OH.$$

From the action of bromine-water upon phenol a yellowish-white crystalline precipitate of tribromophenol results:

$$C_6H_5.OH + 6Br = 3HBr + C_6H_2Br_3.OH.$$

As 331 (molecular weight) parts by weight of tribromophenol correspond to 94 (molecular weight) parts by weight of phenol, the amount of the latter contained in a certain volume of urine is readily determined according to the equation

$$331 : 94 :: x : y\ ;\ \text{and}\ y = \frac{94.x}{331} = 0.28398\ x,$$

in which x indicates the weight of the tribromophenol found in the amount of urine employed, and y the corresponding quantity of phenol.

Method.—From 500 to 1000 c.c. of urine are treated with one-fifth of an equivalent amount of dilute hydrochloric acid (1 : 4), and distilled so long as a specimen of the distillate is rendered cloudy upon the addition of bromine-water (1 : 30), the specimens used fo this purpose being carefully preserved. The total quantity of the filtered distillate, together with the specimens which have been set aside, is now treated with bromine-water, shaking the mixture after each addition of the reagent until a permanent yellow color results. Beyond this point further addition is beset with danger, as compounds will be formed which contain more bromine, the presence of which would indicate a smaller amount of phenol than that actually contained in the urine. After two or three days the precipitate is collected on a filter which has been dried over sulphuric acid, washed with water containing a trace of bromine, and then dried over sulphuric acid and weighed.

Pyrocatechin.—Urines containing pyrocatechin, like those containing hydrochinon (see above), darken upon standing, though presenting a normal color when voided.

ACETONE.

The amount of acetone which may be found in the urine under normal conditions varies between 0.008 and 0.027 gramme, and is greatly influenced by the character of the diet. Whenever the carbohydrates are withdrawn the quantity rapidly increases, and reaches its maximum about the seventh or eighth day. At this time from 200 to 700 mgrms. may be eliminated in the twenty-four hours. If, then, carbohydrates are again added to the diet, the acetonuria soon disappears. This result is not reached, however, if fats are substituted for the carbohydrates. The acetonuria is greatest when but little albuminous food and no carbohydrates at all are ingested, and during starvation the same amounts are essentially found. There can hence be no doubt that acetone is derived from proteid material. Increased amounts are accordingly found whenever, as in fevers, the various cachexias, in conditions associated with inanition, etc., large quantities of circulating albumin are broken down, or whenever carbohydrates are not furnished in sufficient amount.[1]

Some other observers have recently attempted to show that the fat is the source of the acetone, but it seems to me that even though fat *may be* its source it is certainly not its only source, and there is indeed more evidence available to show that acetone may be derived from carbohydrates, if we are to consider sources outside of the albumins.[2] Blumenthal and Neuberg have succeeded in obtaining acetone directly from gelatin by oxidation, so that its possible origin from this source at any rate can be regarded as established.

Most important is the diabetic form of acetonuria, and it may be stated, as a general rule, that the diagnosis of diabetes mellitus is justifiable whenever sugar and notable quantities of acetone are found in the urine. The amount of acetone, moreover, stands in a direct relation to the intensity of the disease, the maximum excretion being usually observed toward the fatal end.[3] Whether or not this form of acetonuria can always be explained upon the basis given above remains an open question. There can be no doubt, however, that the threatening symptoms which are so commonly associated with a greatly increased elimination of acetone will often disappear when carbohydrates are administered in large amounts. It is certain, moreover, that diabetic coma is more apt to occur when the old-fashioned plan of excluding carbohydrates entirely from the dietary of diabetic patients is adopted. Hirschfeld[4] suggests that in every

[1] v. Jaksch, Ueber Acetonurie u. Diaceturie, Hirschwald, Berlin, 1885. Rosenfeld, Centralbl. f. inn. Med., 1895, vol. xv. Waldvogel, "Zur Lehre von der Acetonurie," Zeit. f. klin. Med., vol. xxxviii. p. 506.

[2] C. Neuberg u. F. Blumenthal, Zeit. f. chem. Phys. u. Pathol., 1902, vol. ii. p. 238.

[3] v. Jaksch, Zeit. f. klin. Med., 1885, vol. x. p. 362. Lorenz, Ibid., 1891, vol. xix. p. 19.

[4] F. Hirschfeld, "Beobachtungen über d. Acetonurie u. das Coma diabeticum," Zeit. f. klin. Med., vol. xxviii. p. 176, and vol. xxxi. p. 212.

case of diabetes the excretion of acetone be carefully followed, and that large amounts of carbohydrates be administered whenever the acetonuria approaches a dangerous extent. This agrees with my experience.

Of the febrile diseases in which acetonuria has been observed may be mentioned typhoid fever, pneumonia, scarlatina, measles, acute miliary tuberculosis, acute articular rheumatism, and septicæmia. In those of short duration, on the other hand, even if the fever is high, as in acute tonsillitis, intermittent fever, the hectic fever of phthisis, etc., an increased elimination of acetone is rarely observed. In the continued fevers the acetonuria is largely referable to the character of the diet, as carbohydrates are usually excluded entirely, and I have repeatedly observed that a return to the normal occurred as soon as sugar was administered in amounts varying from 50 to 100 grammes.

In certain nervous and mental diseases, as in general paresis, melancholia, following epileptic seizures, and in tabes, acetonuria is frequently observed. During the second stage of general paresis increased amounts are indeed quite constantly found, but Hirschfeld is probably correct in stating that the psychotic form of acetonuria is largely referable to improper feeding.

In the primary diseases of the stomach, and notably in carcinoma, acetonuria is frequently observed, and it is possible that the acetone in these cases is to some extent at least formed in that organ directly from the proteids ingested. The fact that in carcinoma acetone may be observed at a time when marked loss of flesh has not as yet occurred, and that larger amounts of acetone may be found in the stomach than in the urine, is certainly in favor of this view.[1]

An enterogenic form of acetonuria has further been described, and it has been urged that in these cases the acetone is referable to the formation of unusually large amounts of fatty acids. Acetonuria of this type is also observed following the ingestion of fatty acids as such (alimentary form). The cases of so-called asthma acetonicum probably belong to this class.[2]

Acetonuria has further been observed early in the course of acute phosphorus poisoning, and may persist throughout, apparently without being an index of the severity of the case.

After chloroform narcosis the condition is also not uncommon.

Tests for Acetone.—Legal's Test.[3]—This test may be applied to the freshly voided urine, but is not conclusive. Several cubic centimeters of urine are treated with a few drops of a strong solution of sodium nitroprusside and sodium hydrate; the mixture assumes a red color, which rapidly disappears, and in the presence of acetone

[1] H. Lorenz, loc. cit.

[2] Waldvogel u. Hagenberg, "Ueber alimentäre Acetonurie," Zeit. f. klin. Med., 1900, vol. xiii. p. 443.

[3] Le Nobel, Arch. f. exper. Path. u. Pharmakol., 1884, vol. xviii. p. 9.

is replaced by a purple or violet red when acetic acid is added. As a rule, it is better to distil the urine (500–1000 c.c.) after the addition of a little phosphoric acid (1 gramme pro liter), and to employ the first 10–30 c.c. of the distillate for one or more of the following tests.

Lieben's Test.[1]—A few cubic centimeters of the distillate are treated with several drops of a dilute solution of iodo-potassic iodide and sodium hydrate, when in the presence even of traces of acetone a precipitation of iodoform in crystalline form occurs, which may be readily recognized by its odor when the solution is heated.

Reynolds' Test.[2]—A few cubic centimeters of the distillate are treated with a small amount of freshly precipitated yellow mercuric oxide. This is prepared by precipitating a solution of mercuric chloride with an alcoholic solution of sodium hydrate. If acetone is present, a black color, due to the formation of mercuric sulphide, will result in the clear filtrate upon the addition of a few drops of ammonium sulphide.

Stock's Test (modified by Frohner).—A crystal of hydroxylamin hydrochlorate is dissolved in about 5 c.c. of the distillate; the solution is treated with hypochlorite of calcium solution and extracted with a little ether. A blue color is obtained, which can still be discerned in the presence of but 0.001 gramme of acetone. The reaction is proof positive of the presence of a ketone, but is for this reason also obtained with diacetic acid.

As originally proposed by Stock, 10 c.c. of the liquid to be examined are treated with 1 drop of a 10 per cent. solution of hydroxylamin, 1 drop of a 5 per cent. solution of sodium hydrate, and a larger drop of pyridin, after which the ether (about 1 c.c.) is added, as also bromine-water while shaking, until the ether has assumed a yellow tint. On the subsequent addition of hydrogen peroxide the yellow changes to blue.

Dennigès' Test (as modified by Oppenheimer).[3]—The reagent is prepared as follows: 20 grammes of concentrated sulphuric acid are poured into 100 c.c. of distilled water, when 5 grammes of freshly prepared yellow mercuric oxide (see Reynolds' test) are added. The mixture is allowed to stand for twenty-four hours and is then ready for use.

This reagent is added to about 3 c.c. of urine, drop by drop, until the precipitate which is thus formed no longer disappears on stirring. When this point is reached a few more drops are added. After two to three minutes the precipitate is filtered off. The clear filtrate is further treated with about 2 c.c. of the reagent, and 3–4 c.c. of a 30 per cent. solution of sulphuric acid, and boiled for a minute or two, or, still better, placed in a vessel with boiling water.

[1] Taniguti u. Salkowski, Zeit. f. physiol. Chem., 1890, vol. xiv. p. 476.
[2] Gunning, Jour. de Pharmacol. et de Chim., 1881, vol. iv. p. 30.
[3] Oppenheimer, Berlin. klin. Woch., 1899, p. 828.

In the presence of an abundant amount of acetone a copious white precipitate forms immediately; while in the presence of traces only (less than 1 : 50000), a slight cloud develops on standing for several minutes. The precipitate is almost entirely soluble in an excess of hydrochloric acid.

If albumin is present, the urine becomes turbid at once when the reagent is added. In that case the test is continued as described, attention being directed to the coarser precipitate which occurs later. To such urines large amounts of the reagent must be added, the idea being to precipitate everything that can be precipitated with the reagent, before heating.

It will be observed that Dennigès' test is much simpler than the tests already described, and Oppenheimer claims that it is as delicate as that of Lieben, viz., giving a well-pronounced reaction with a dilution of 1 : 20000, and being still discernible with a dilution of 1 : 60000. As diacetic acid yields acetone when treated with mineral acids, a positive result is always obtained when this is present. But as diacetic acid is usually found only in association with acetone, this fact does not lessen the value of the test, and is an error, moreover, which is common to the other tests as well.

Quantitative Estimation of Acetone.—For the purpose of estimating the amount of acetone the method of Messinger, as modified by Huppert, is now employed, and is greatly to be preferred to the older procedure of v. Jaksch.[1]

Principle.—The method is based upon the observation of Lieben that acetone gives rise to the formation of iodoform when treated with iodine in an alkaline solution. If, then, a solution of acetone is treated with a known amount of iodine, it is a simple matter to determine the quantity present by retitrating the iodine which was not used in the formation of iodoform.

Solutions required:

1. Acetic acid (50 per cent. solution).
2. Sulphuric acid (12 per cent. solution).
3. Sodium hydrate solution (50 per cent.).
4. A decinormal solution of iodine.
5. A decinormal solution of sodium thiosulphate.
6. Starch solution (see page 252).

Preparation of the solutions:

1. The decinormal solution of iodine is prepared as described elsewhere (see page 251).

2. As the molecular weight of sodium thiosulphate—$Na_2S_2O_3 + 5H_2O$—is 248, a decinormal solution of the salt would contain 24.8 grammes to the liter. This quantity is dissolved in about 950 c.c. of distilled water, and brought to the proper strength by titration

[1] See Neubauer u. Vogel, Analyse des Harns, 9th ed., p. 470.

with a decinormal solution of iodine. As 1 c.c. of the thiosulphate solution should correspond to 1 c.c. of the iodine solution, the necessary amount of water which must be added to the former is then determined.

Method.—One hundred c.c. of urine, or less if much acetone is present, as determined by Legal's test, are treated with 2 c.c. of the acetic acid solution and distilled until seven-eighths of the total amount have passed over. The distillate is received in a retort which is connected with a bulb-tube containing water. As soon as seven-eighths of the urine have distilled over, a small amount of the distillate of the remainder is tested for acetone according to Lieben's method. Should a positive reaction be obtained, it will be necessary either to repeat the entire process with less urine, diluted to about 200 c.c., or to add about 100 c.c. of water to the residue and to distil until all the acetone has passed over. The distillate is then treated with 1 c.c. of the sulphuric acid and redistilled. The addition of the acetic acid and of the sulphuric acid, respectively, serves the purpose of holding back phenol and ammonia. Should the first distillate contain nitrous acid, moreover, which is recognized on the addition of a little starch paste containing a trace of potassium iodide, when the solution turns blue, the acid is removed by adding a little urea. The second distillate is received in a bottle provided with a well-ground glass stopper, and holding about 1 liter. The distillate is then treated with a carefully measured quantity of the one-tenth normal solution of iodine,—about 10 c.c. for 100 c.c. of urine,—and sodium hydrate solution until the iodoform separates out. To this end, a slight excess of the solution must be added. Should ammonia be present, a blackish cloud will be observed at the zone of contact of the sodium hydrate and the iodine solution, and it will be necessary to repeat the entire process. The bottle is closed and shaken for about one minute. The solution is then acidified with concentrated hydrochloric acid, when the mixture assumes a brown color if iodine is present in excess. If this does not occur, more of the iodine solution must be added, and the process repeated until an excess is present. The excess is then retitrated with the thiosulphate solution until the fluid presents a faint-yellow color. A few cubic centimeters of starch solution are now added, and the titration continued until the last trace of blue has disappeared. The number of cubic centimeters employed in the titration is finally deducted from the total amount of the iodine solution added, and the result multiplied by 0.976. The figure thus obtained indicates the amount of acetone contained in the 100 c.c. of urine, in mgrms., as 1 c.c. of the thiosulphate solution is equivalent to 1 c.c. of the iodine solution, or to 0.967 mgrm. of acetone.

DIACETIC ACID.

The occurrence of diacetic acid in urine must always be regarded as abnormal. Its pathological significance is identical with that of acetonuria. It is met with especially in diabetes, in various digestive diseases, and in febrile diseases. In the continued fevers of childhood it is almost constantly present.

Gerhardt's Test.—In order to demonstrate the presence of diacetic acid a few cubic centimeters of urine are treated with a strong solution of ferric chloride added drop by drop. A precipitate of phosphates is filtered off, when more of the iron solution is added to the filtrate. If now a Bordeaux-red color appears, another portion of the urine is boiled and similarly treated. If in the second test no reaction is obtained, a third portion of the urine is treated with sulphuric acid and extracted with ether. A positive reaction, when the ethereal extract is tested with ferric chloride, the color disappearing upon standing for twenty-four to forty-eight hours, will indicate the presence of diacetic acid, particularly if the urine is rich in acetone. With Gerhardt's test a negative reaction is obtained even though diacetic acid be present, if the patient has been taking salol, viz., when salicylic acid is being excreted by the urine. In such case the urine should be filtered through animal charcoal, which retains the salicylic acid, but does not interfere with the diacetic acid.

Arnold's Test.—Two solutions are employed, viz., a solution of para-amido-aceto-phenon and a 1 per cent. solution of sodium nitrite. The first is prepared by dissolving 1 gramme of para-amido-aceto-phenon in from 80 to 100 c.c. of distilled water, and adding hydrochloric acid drop by drop until the solution, which at first is yellow, becomes colorless; an excess, however, should be avoided. Immediately before using, the two solutions are mixed in the proportion of two to one. A few cubic centimeters of the reagent are then treated with an equal volume of urine, and a few drops of ammonia added. Thus treated, all urines give a more or less marked brownish-red color on shaking; and if much diacetic acid is present, an amorphous reddish-brown sediment is thrown down. A small amount of the colored solution is then placed in a conical glass and treated with an excess of concentrated hydrochloric acid (10–12 c.c. for each 1 c.c.). In the presence of diacetic acid the mixture assumes a beautiful purplish-violet color.

According to Arnold, the test is more delicate than that of Gerhardt, and does not respond with acetone or oxybutyric acid. With bilirubin and the common antipyretics, as well as salicylic acid, no reaction is obtained. Highly colored urines should first be filtered through animal charcoal.

According to Lipliawski, the following modification of Arnold's

test is even more sensitive: two solutions are employed, viz., a 1 per cent. solution of para-amido-aceto-phenon and a 1 per cent. solution of potassium nitrite. Six c.c. of the first solution and 3 c.c. of the second are added to an equal volume of urine, together with a drop of concentrated ammonia. The mixture is shaken until it assumes a brick-red color. From 10 drops to 2 c.c., according to the amount of diacetic acid present, are treated with 15–20 c.c. of concentrated hydrochloric acid (sp. gr. 1.19), 3 c.c. of chloroform, and 2–4 drops of an aqueous solution of ferric chloride. The tube is closed with a cork and *gently* agitated (so as to avoid emulsification), when after one-half to one minute a beautiful and very characteristic violet tinge results if diacetic acid is present. In its absence the color is yellowish or slightly reddish. The violet color persists for a long time. Salicylic acid, phenacetin, antipyrin, phenol, and other drugs are without disturbing influence upon the reaction.

Allard states that both Arnold's test and that of Lipliawski give a positive result also with acetone, when this is present to the extent of more than 1 per cent.

LITERATURE.—v. Jaksch, Ueber Acetonurie u. Diaceturie, loc. cit. Idem., Zeit. f. Heilk., 1882, vol. iii. p. 34. Schrack, Jahrbuch f. Kinderheilk, 1889, vol. xxix. p. 411. V. Arnold, Wien. klin. Woch., 1899, p. 541.

OXYBUTYRIC ACID.

The fact that in some cases of diabetes an excessive elimination of ammonia was observed led to the belief that there must be present an unknown acid; this was shown to be β-oxybutyric acid. The occurrence of this acid in the urine of diabetic patients is of great clinical interest, not only from the standpoint of diagnosis, but also of prognosis and treatment. Its presence may always be regarded as indicating a severe type of the disease, and when associated with marked acetonuria and diaceturia as indicating the possible occurrence of coma.

According to Herter, the condition of diabetic coma is preceded by a period of days, weeks, or months, in which there is a large excretion of β-oxybutyric acid (20 grammes or more in twenty-four hours), and in which the nitrogen of ammonia is largely increased. The same writer states that patients whose urines show or have shown a large excretion of organic acids are in danger of developing diabetic coma; but the nitrogen of ammonia may temporarily rise as high as 16 per cent., and yet coma may be delayed for more than seven months. The persistent excretion of more than 25 grammes of β-oxybutyric acid indicates impending coma. Important also is the observation that while as a general rule the appearance of large amounts of organic acids is associated with the presence

of much sugar, a constant relation between the two does not exist. There may thus be much sugar and little or no acid in the urine, or there may be much acid and little sugar.

The presence of oxybutyric acid may be inferred in diabetic urines if after fermentation a rotation of the plane of polarization to the left is observed.

Quantitative Estimation (according to Darmstaedter).—The method is based on the decomposition of the β-oxybutyric acid with the formation of α-crotonic acid and the estimation of the latter. This decomposition takes place according to the equation:

$$\underset{\beta\text{-oxybutyric acid.}}{CH_3.CHOH.CH_2.COOH} = \underset{\text{Crotonic acid.}}{CH_3.CH.CH.COOH} + H_2O.$$

100 c.c. of urine are rendered feebly alkaline with sodium carbonate and evaporated on a water-bath almost to dryness. With the aid of 150–200 c.c. of sulphuric acid (50–55 per cent.) the residue is transferred to a litre flask, which is closed with a doubly perforated stopper. Through the one aperture a drip-tube passes, while a bent glass tube passes through the other to a condenser. Heat is applied, at first mildly, so as to avoid foaming; then vigorously. Water is allowed to enter through the drip-tube as fast as the distillate passes over. The distillation is interrupted when from 300 to 350 c.c. have been obtained, which usually takes from two to two and one-half hours. The distillate is extracted two or three times with ether. The ether is distilled off, the residue heated for a few minutes on a sand-bath to 160° C. in order to drive off any fatty acids that may be present, and then dissolved on cooling with 50 c.c. of water. The solution is filtered, and the filter washed with a little water. The aqueous solution of the crotonic acid is now titrated with a decinormal sodium hydrate solution, using phenolphthalein as an indicator. 1 c.c. of the soda solution corresponds to 0.0086 gramme of crotonic acid. The corresponding amount of oxybutyric acid is obtained by multiplying by 1.21. Sugar does not interfere with the process.

If it is only desired to prove the presence of oxybutyric acid in the urine, this method can also be conveniently employed. The crotonic acid is obtained from the ethereal extract, and recognized by its melting-point, 72° C. If necessary, it can be purified by solution in water and re-extraction with a small amount of ether and subsequent evaporation, viz., distillation of the ether.

LITERATURE.—v. Jaksch, Ueber Acetonurie u. Diaceturie, loc. cit. H. Wolpe, Arch. f. exper. Path. u. Pharmakol., 1886, vol. xxi. p. 131. Herter, "The Acid Intoxication of Diabetes in its Relation to Prognosis," Jour. of Exper. Med., 1901, vol. v. p. 617. E. Darmstaedter, Zeit. f. phys. Chem., 1903, vol. xxxvii. p. 355.

CROTONIC ACID.

As has just been shown, crotonic acid is a derivative of oxybutyric acid. Its presence in the urine as such has not as yet been established, and it is likely that statements to the contrary are based upon findings of the acid in the distillate, especially when the distillation has been carried on after the addition of sulphuric acid to the urine. But even in the absence of a free acid a small amount of crotonic acid results from oxybutyric acid on boiling.

LACTIC ACID.

Sarcolactic acid is normally absent from the urine, but is met with in pathological conditions, and particularly in hepatic diseases, as the liver is normally concerned in the decomposition of lactic acid and of the lactates that have been ingested with the food. As has been pointed out, moreover, there is evidence to show that by far the greatest portion of the nitrogen eliminated from the body reaches the liver as ammonium lactate, and is here synthetically transformed into urea. As a consequence, lactic acid appears in the urine whenever, as in phosphorus poisoning, acute yellow atrophy, etc., an extensive destruction of the hepatic parenchyma occurs, and the formation of urea is consequently impaired. In such cases the elimination of lactic acid is associated with an increased excretion of ammonia. The same will occur when, owing to insufficient oxygenation of the blood, the power of oxidation on the part of the liver is interfered with. We accordingly find lactic acid in the urine in the chronic anæmias, in cases of poisoning with carbon monoxide, in association with the various forms of circulatory and respiratory dyspnœa, in cases of epilepsy immediately after the attack, following excessive muscular exercise, as in soldiers after forced marches, etc.

In order to test for lactic acid, the urine is evaporated on a water-bath to a thick syrup and extracted with 95 per cent. alcohol. This is decanted off after twenty-four hours, evaporated to a syrup, acidified with dilute sulphuric acid, and extracted with ether so long as this presents an acid reaction. The ether is then distilled off and the residue dissolved in water. This solution is treated with a few drops of a solution of basic lead acetate, filtered, the excess of lead removed by means of hydrogen sulphide, and the filtrate evaporated to dryness on a water-bath, when the lactic acid will remain behind as a slightly yellowish syrup. This is then dissolved in a little water, the solution is saturated with zinc carbonate, and boiled. Zinc lactate will separate out upon evaporation, especially if a little alcohol is added, and may be recognized by the form of its crystals, viz., small prisms. These crystals are lævorotatory, soluble in alcohol (1 : 1100), and contain two molecules of water of crystallization,

which is lost at 105° C., so that the loss of weight after heating to this temperature must correspond to 12.9 per cent.

LITERATURE.—O. Minkowski, "Ueber den Einfluss d. Leberextirpation auf d. Stoffwechsel," Arch. f. exper. Path. u. Pharmakol., vol. xxi. p. 41; and "Ueber Ursache d. Milchsäureausscheidung nach Leberextirpation," Ibid., vol. xxxi. p. 214. G. Colosanti u. R. Moscatelli, "Ueber d. Milchsäuregehalt d. menschlichen Harns, Ibid., vol. xxvii. p. 158. Jnouye and Saiki, "Lactic Acid after Epileptic Attacks," Zeit. f. physiol. Chem., 1903, vol. xxxvii. p. 203.

OXYAMYGDALIC ACID.

Schultzen and Riess[1] discovered an acid in the urine of patients who had died from acute yellow atrophy to which they gave the formula $C_8H_8O_4$. They regard it as oxyamygdalic acid and suppose it to be derived from tyrosin, which was also found, according to the equation :

$$C_9H_{11}NO_3 + 3O = CO_2 + NH_3 + C_3H_8O_4.$$

Very curiously it was not found in cases of phosphorus-poisoning, but only in acute yellow atrophy. As in this disease there is coincidently with the rapid parenchymatous destruction much extravasation of blood, Nencki has suggested that the acid in question may possibly be derived from blood-pigment, especially as Küster obtained from hæmatoporphyrin an acid which has the formula $C_8H_8O_5$, and which thus only differs from the product of Schultzen and Riess by a plus of one atom of oxygen.

VOLATILE FATTY ACIDS.

The term *lipaciduria* is applied to the elimination of volatile fatty acids in the urine. This occurs under normal conditions, but may be much more marked in disease. With an ordinary diet the degree of lipaciduria corresponds to from 50 to 80 c.c. $\frac{1}{10}$ normal sulphuric acid. In febrile conditions, according to v. Jaksch and Rokitansky, there is an increase, which runs parallel to the height of the temperature. Rosenfeld, however, has shown that this is, strictly speaking, not correct, and that an increase is only observed in those febrile states in which resorption of breaking down albuminous material is taking place, as in cases of tonsillar abscess, septic diphtheria, putrid bronchitis, and empyema, and in general in association with all suppurative processes and hemorrhages within the body. Especially high values are found during convalescence from pneumonia, during the first days following crisis. This is no doubt owing to a resorption of the exudate, and is associated with an increased elimination of nitrogen. Immediately before the crisis it is common to meet with very low values—20 c.c.—as compared

[1] O. Schultzen u. L. Riess, Annalen d. Charité Krankenhauses zu Berlin, 1869, vol. xv.

with 100–240 c.c. during convalescence. These observations, as Rosenfeld has pointed out, may be of marked value in the diagnosis of obscure accumulations of pus.

A marked decrease in the amount of fatty acids is noted in uncomplicated cases of erysipelas and scarlatina (30–50 c.c.), in measles, diphtheria, and, as I have already indicated, in pneumonia preceding active resorption of the exudate (20–40 c.c.).

According to some observers, the amount of fatty acids in the urine may be regarded as an index of the degree of carbohydrate fermentation in the intestinal tract. Under normal conditions this may be the case, but in disease the question is probably more complicated.

The acids in question are formic acid, acetic acid, butyric acid, and propionic acid. They may be isolated as described in the chapter on the Feces.

For their *quantitative estimation* it will suffice to distil a given volume of urine with sulphuric acid and to titrate the distillate with $\frac{1}{10}$ normal sodium hydrate solution. The results are expressed in terms of the number of c.c. of $\frac{1}{10}$ normal sulphuric acid corresponding. 250 c.c. of the urine, which must be fresh or preserved with chloroform, are distilled with 50 c.c. of dilute sulphuric acid until 200 c.c. have passed over. The residue is diluted with 200 c.c. of water and the distillation continued as before. In this manner the danger that some hydrochloric acid may pass over is avoided, but it is well to make sure of this by testing with silver nitrate.

The method is exact; traces of benzoic acid are included, but in man these can be neglected.

LITERATURE.—v. Jaksch, Zeit. f. klin. Med., 1886, vol. xi. p. 307; and Zeit. f. physiol. Chem., 1886, vol. x. p. 536.

FAT.

Under strictly normal conditions the urine contains no fat, while variable amounts may be found in disease. When present in large quantities, so that it is possible to recognize it with the naked eye, the condition is termed *lipuria*. Such cases, however, are rare, and the diagnosis should only be made when it is possible to exclude an accidental contamination of the urine. Smaller quantities of fat, recognizable only with the microscope, are much more common, and are indeed quite constantly observed whenever fatty degeneration of the renal epithelial cells, of pus-corpuscles, or of tumor-particles is taking place in the urinary tract. The fat-droplets may then be found floating in the urine or attached to or imbedded in any morphological elements that may be present. Lipuria may also occur when abnormally large quantities of fat are circulating in the blood. It is

thus observed after the administration of cod-liver oil in large quantities, following oil inunctions, in cases of fracture of the long bones with extensive destruction of the bone-marrow, in cases of eclampsia, as also in such diseases as diabetes mellitus, chronic alcoholism, phthisis, obesity, leukæmia, in certain mental diseases, in affections of the pancreas and heart, etc.

The term *chyluria* or *galacturia* has been applied to a condition in which a turbid urine presenting the macroscopical appearance of milk is excreted. Upon microscopical examination it may be demonstrated that the turbidity in such cases is owing to the presence of innumerable highly refractive globules of fat, which may be removed by shaking with ether. Of morphological constituents, leucocytes are occasionally encountered in large numbers. Red blood-corpuscles are also seen at times, and when present in large numbers impart a rose color to the urine. Fibrinous coagula are often observed when the urine has stood for some time, and the entire bulk of urine may even become transformed into a gelatinous mass. Albumin is present in most cases in the absence of other constituents pointing to renal disease, such as tube-casts and renal epithelial cells. Leucin, tyrosin, and cholesterin may also at times be found, particularly the latter. It has been quite generally accepted that chyluria is due to the presence of the Filaria sanguinis hominis; but while filariæ are undoubtedly present in the blood in the majority of instances, and may also be present in the urine, it has been demonstrated that cases occur in which filariasis does not exist, and Götze expressed the opinion that chyluria may be owing to a distinct anatomical lesion affecting the renal parenchyma.

Literature.—Lipuria: Schütz, Prag. med. Woch., 1882, vol. vii. p. 322. Ebstein, Arch. f. klin. Med., 1879, p. 115. Chyluria: Huber, Virchow's Archiv, 1886, vol. cvi. p. 126. Rossbach-Götze, Verhandl. d. Congr. f. inn. Med., 1887, vol. vi. p. 212. Brieger, Zeit. f. physiol. Chem., 1880, vol. iv. p. 407. Grim, Langenbeck's Archiv, 1885, vol. xxxii. p. 511.

FERMENTS.

Ferments may be demonstrated in every urine, both under physiological and pathological conditions. Pepsin (viz., a proteolytic ferment) is said to be absent in cases of typhoid fever, carcinoma of the stomach, and possibly also in nephritis. In order to demonstrate its presence, a small flake of boiled fibrin is placed in the urine, and after several hours removed to a 2 to 3 pro mille solution of hydrochloric acid. The pepsin, if present, will be deposited upon the fibrin and effect digestion of the latter in the hydrochloric acid solution. Diastase, a milk-curdling ferment, a fat-splitting ferment, and a ferment causing decomposition of urea into carbon dioxide and ammonia, have also been observed.

It is noteworthy that the fat-splitting ferment was encountered

in a case of hemorrhagic pancreatitis, and it has been suggested that its presence may possibly be of value in the diagnosis of the disease. Opie, who reports the case, demonstrated its presence by the method of Castle and Loevenhart. Only a small amount of urine was obtained. This was neutralized with potassium hydroxide and divided into two portions. To one portion were added a few drops of carefully purified ethyl butyrate together with a small quantity of litmus solution. The second portion used as a control was boiled in order to destroy the ferment if present, and ethyl butyrate added. Both specimens were kept at 37° C.; at the end of twenty-four hours the unboiled specimen had acquired a well-marked acid reaction, while the control specimen was little if at all changed.

Since the diagnosis of acute lesions of the pancreas is difficult and at times impossible the demonstration of the constant occurrence of the ferment under such circumstances would be of great importance. Future investigations in this direction are urgently needed.

LITERATURE.—Opie, Johns Hopkins Hospital Bull., 1902, vol. xiii. p. 117; Castle and Loevenhart, Am. Chem. Jour., vol. xxiv.

GASES.

Every urine contains a small amount of gases, notably carbon dioxide, oxygen, and nitrogen, which may be withdrawn by means of an air-pump.

Under pathological conditions hydrogen sulphide is at times also found, constituting the condition known as *hydrothionuria*. In some instances this is referable to a diffusion of the gas into the bladder from neighboring organs or accumulations of pus; but this is rare. In others an abscess has ruptured into the bladder, or a direct communication exists between it and the bowel. Under such conditions it can, of course, not be surprising that hydrogen sulphide together with other products of albuminous putrefaction are eliminated in the urine. More commonly, however, the hydrothionuria occurs idiopathically, and is then referable to the action of certain micro-organisms. This can be readily demonstrated by adding a few cubic centimeters of such urine to normal urine, when upon standing the formation of hydrogen sulphide may be demonstrated in the normal specimen. The common organisms, however, which cause ammoniacal decomposition apparently have no part in this process, and the formation of the hydrogen sulphide may be observed before ammoniacal decomposition has set in and while the reaction is yet acid. If a small amount of ordinary decomposing urine, moreover, is added to fresh normal urine, no hydrogen sulphide is as a rule produced. The character of the organisms in question is variable; sometimes micrococci are found, at other times bacilli, and in still other instances both. Besides being capable of

producing hydrogen sulphide from the sulphur bodies of the urine, some of them also cause the formation of ammonium carbonate in dilute solutions of urea.

The source of the hydrogen sulphide in cases of hydrothionuria is in most cases probably the so-called neutral sulphur, but it is possible that the oxidized sulphur is at times also attacked. Very interesting is the fact that in cystinuria, in which the neutral sulphur is more or less increased, hydrothionuria is commonly observed. Its occurrence in such cases is indeed so frequent that I am inclined to suspect cystinuria, although crystals of cystin are not found in the sediment. Further work in this direction, however, is needed, and especially to determine the relative frequency with which the two conditions are associated.

In a few recorded instances the hydrothionuria accompanied indigosuria, viz., the presence of free indigo-blue in the urine; and this Müller has likewise shown to be referable to the action of certain micro-organisms (see page 627). One case of this kind I saw several years ago, but made no examination for the presence of cystin.

Owing to the well-known poisonous effect of hydrogen sulphide upon the blood, it is well in every case to ascertain whether its formation occurs in the bladder, or whether it takes place only on standing. The formation of hydrogen sulphide in decomposing urines containing albumin is, of course, common, and should not be confused with the idiopathic hydrothionuria here described.

The chemical test for hydrogen sulphide is very simple: a strip of filter-paper is moistened with a few drops of sodium hydrate and lead acetate solution and clamped into the neck of the bottle containing the urine. After a variable length of time, in some instances immediately, in others only after twelve to twenty-four hours, a discoloration of the paper will be observed, varying from a grayish brown to black according to the amount present. When this is large it is, of course, also recognized by its characteristic odor.

LITERATURE.—F. Müller, "Schwefelwasserstoff im Harn," Berlin. klin. Woch., 1887, Nos. 23 and 24. Rosenheim u. Gutzmann, Deutsch. med. Woch., 1888, No. 10. Kahler, Prag. med. Woch., 1888, No. 50.

PTOMAÏNS.

Numerous researches have shown that traces of toxic alkaloidal substances may be encountered in the urine under the most diverse pathological conditions, and may be present even in health. Of the nature of these bodies, however, little is known. Thudichum claims to have isolated three distinct basic substances from normal urine, which he has termed *reducin*, *parareducin*, and *aromïn*. Pouchet and Mme. Eliacbeff, working in Gautier's laboratory, have likewise extracted toxic bodies from normal urines; and Adduco

states that after fatiguing exercise, especially, he could demonstrate in the urine a substance which was extremely toxic, and was not identical with cholin, as was first supposed. All this work, however, must be repeated with great care before the results obtained can be regarded as conclusive. This is also true of the work which has been done in various diseases. Some observers have here described bodies which they regard as specific toxins. Griffith thus reports the presence of a specific poison of scarlatina, of measles, mumps, etc. Others again have obtained only negative results.

The only substances belonging to the class of ptomaïns which have thus far been obtained from the urine in amounts sufficient to establish their identity are *cadaverin* and *putrescin*. They were originally discovered by Brieger in putrefying cadavers, and subsequently also found in cultures of the bacillus of Asiatic cholera, the Finkler-Prior bacillus of cholerina, the bacillus of tetanus, and in the rice-water stools of cholera patients. From the urine cadaverin, putrescin, and a third diamin isomeric with cadaverin, and which has been regarded as saprin or neuridin, were first obtained by Baumann and v. Udranszky in a case of cystinuria, and it appears that diaminuria occurs only in association with this disease. All attempts to isolate diamins from the urine under other pathological conditions at least have given rise to negative results. Whether or not diaminuria is invariably associated with cystinuria is, however, an open question. Putrescin has thus far been found in only three cases, viz., in the first case of Baumann and v. Udranszky, in Bödtker's case, and in a recent, as yet unpublished, case by Garrod. Brieger, Stadthagen, Leo, Garrod, Lewis, and I have succeeded in isolating cadaverin from such urines. Others have been less successful, and the theory which was announced shortly after Baumann's discovery, and quite generally accepted, namely, that the formation of the diamins in question is in some manner responsible for the appearance of cystin in the urine, was certainly premature. This is even more true of the inference drawn from this supposed association, viz., that cystinuria is a specific infectious disease of the intestinal canal. This conclusion was based upon the belief that diamins are formed from albuminous material only in the presence of certain bacteria. I have shown, however, that this is not necessarily the case, and that putrescin at least may be formed in the absence of micro-organisms. Further investigation will show whether or not cystinuria is invariably accompanied by diaminuria. Personally I incline to the belief that this is the case; but I have also shown that while cystinuria and diaminuria may coexist, this is not always so, and that the two conditions may alternate, and that the one may temporarily disappear while the other continues. Like Moreigne, I have been led to the conclusion that diaminuria is a metabolic anomaly analogous to diabetes and gout, and that both

diaminuria and cystinuria are the expression of a marked impairment of the normal oxidation-processes of the body.

The amount of diamins which may be met with in the urine of cystinuric patients is extremely variable. In one case I was able to isolate as much as 1.6 grammes of the benzoylated cadaverin from the collected urine of twenty-four hours.[1] On other days traces only were present, and at times, as I have already stated, no diamins at all could be found. A few observers who have investigated this question, state that they were unable to find even traces of diamins in their cases; but as single examinations only were made, their conclusion that diaminuria does not always accompany cystinuria is scarcely justifiable. When single negative results are obtained, the examination should be repeated at frequent intervals or larger quantities of urine employed. In general, I should advise those who wish to investigate the question of ptomaïnuria to experiment with large quantities of urine only, as some of the bodies belonging to this order exhibit a degree of toxicity which is out of all proportion to the amount present. Where specific alkaloids are to be sought for, it is scarcely worth while to use less than 100 or 200 liters of urine, and even with such amounts the results are frequently disappointing. In cases of cystinuria much smaller quantities will usually suffice, and an initial experiment may be made with the collected urine of twenty-four hours.

Isolation of Diamins.—Method of Baumann and v. Udranszky.—The collected urine of at least twenty-four hours is shaken with a 10 per cent. solution of sodium hydrate and benzoyl chloride in the proportion of 1500 : 200 : 25 until the odor of the benzoyl chloride has entirely disappeared. The resulting precipitate contains phosphates, the benzoyl compounds of the normal carbohydrates of the urine, and a portion of the benzoylated diamins. These are filtered off with the aid of a suction-pump and digested with alcohol. The filtered alcoholic extract is concentrated to a small volume and poured into about 30 times its amount of water. Upon standing for from twelve to forty-eight hours the benzoylated diamins separate out in the milky fluid in the form of a more or less voluminous sediment composed of fine, intensely white crystals. In order to remove the benzoylated carbohydrates likewise present, the precipitate is redissolved in alcohol, the solution concentrated to a small volume, and diluted with water as described. This process is repeated several times. The resulting crystals, if both diamins are present, will lose their water of crystallization at 120° C. and melt at 140° C.

A smaller portion of the benzoyl diamins remains in the first filtrate. In order to recover this, the filtrate is acidified with sulphuric acid and extracted with ether. The ethereal residue, before congeal-

[1] In the case of Dr. Lewis, which was examined in my laboratory, 0.3 gramme only could be obtained from 12,000 c.c.

ing, is placed in as much of a 12 per cent. solution of sodium hydrate as is required for its neutralization, when from 3 to 4 times the volume of the same solution is added. This mixture is placed in the cold, when long needles and platelets separate out, which consist of the sodium compound of benzoyl cystin and the benzoylated diamins. The sediment is filtered off and placed in cold water, in which the sodium-benzoyl cystin dissolves, while the benzoylated diamins remain undissolved.

In order to separate the putrescin from the cadaverin, the crystals are dissolved in a little warm alcohol and treated with 20 times the volume of ether. Benzoyl-putrescin is thus thrown down, and may be recognized by its melting-point, viz., 175°–176° C., while the ethereal residue contains the benzoyl-cadaverin, which melts at from 129° to 130° C.

The diamins may then be separated from the benzoyl radicle by heating the crystals on a water-bath with a mixture of equal parts of alcohol and concentrated hydrochloric acid until a specimen is entirely dissolved by sodium hydrate. The separation is complete after from twenty-four to forty-eight hours, according to the amount present. The solution is then diluted with water, when the benzoic acid, which has been formed, separates out and is filtered off. After extracting with ether, in order to remove any benzoic acid still remaining, the filtrate is evaporated to dryness. A crystalline mass remains, which is easily soluble in water but with difficulty in alcohol. This consists of putrescin and cadaverin hydrochlorates, from which the various double salts with platinum, silver, mercury, etc., can be readily obtained. The platinum salt of cadaverin is formed by adding an alcoholic solution of platinum chloride to a solution of the hydrochlorate in alcohol; it occurs as a voluminous yellow crystalline mass, which can be purified by recrystallization from hot water. When this salt is decomposed by hydrogen sulphide the hydrochlorate again results, from which the free base is obtained by distillation with caustic potash. During this distillation water passes over at first; and above 160° C. a colorless oil appears, the boiling-point of which is about 173° C. This constitutes the free base, which may be identified by its sperm-like odor and the avidity with which it attracts carbon dioxide from the air to form a carbonate.

Literature.—Stadthagen, "Ueber d. Harngift," Zeit. f. klin. Med., 1889, vol. xv. p. 383. Bouchard, Compt. rend. Soc. de Biol., 1884; and Compt. rend. de l'Acad. des Sci., vol. cii. p. 1127. Lépine et Aubert, Ibid., vol. ci. p. 90. Adduco, Arch. ital. d. Biol., vol. ix. p. 203, and x. p. 1.

Diaminuria: v. Udranszky u. Baumann, Zeit. f. physiol. Chem., 1889, vol. xiii. p. 562. Stadthagen u. Brieger, Berlin. klin. Woch., 1889, vol. xxvi. p. 344. Bödtker, Norsk. Mag. f. Laegevidensk., 1892, vol. vii. p. 1220. Moreigne, Arch. de Méd. expér. et d'Anat. path., 1899, p. 254. Simon, Am. Jour. Med. Sci., 1900, vol. cxix. p. 39. Garrod and Cammidge, Jour. Path. and Bact., Feb., 1900.

KRYOSCOPIC EXAMINATION OF THE URINE.

The kryoscopic examination of the mixed urine does not furnish as valuable information as the corresponding examination of the blood. This is largely owing to the fact that the normal variations in the freezing-point of the urine are much more extensive—*i. e.*, between —0.9° and —2° C. In the determination of renal insufficiency, however, where specimens from each kidney separately are available, or at least one specimen for one kidney together with a mixed specimen from the same patient, the method furnishes very satisfactory results; it indicates the location of the disease even more definitely than a quantitative estimation of urea, tests of specific gravity, and the other usual tests of the urine. Especially interesting are the results which are obtained in cases of unilateral disease of the kidneys in which the other organ is functioning normally; kryoscopic examination of the blood will then furnish normal values as there is normal elimination, while a separate examination of the urine from the two sides reveals the diseased kidney. A value of Δ higher than —0.9° C. is abnormal.

The examination is conducted as described in the case of the blood.

LITERATURE.—See page 163.

MICROSCOPICAL EXAMINATION OF THE URINE.

Sediments.

In the chapter treating of the general physical characteristics of the urine it was stated that, on standing, every urine gradually becomes cloudy owing to development of the so-called nubecula. This was shown to consist of a few mucous corpuscles, a small number of pavement epithelial cells derived from the urinary and genital passages, and under certain conditions of a few crystals of uric acid, of calcium oxalate, or of both. It was further pointed out that owing to a diminution in the acidity of the urine on standing, in consequence of an interaction between the neutral sodium urate and the acid sodium phosphate, a sediment is thrown down which consists of acid sodium urate, and at times of free uric acid (see Reaction). Should the reaction of the urine be alkaline, however, when freshly voided, a condition which may occur physiologically, when it is dependent upon the ingestion of large quantities of vegetables rich in organic salts of the alkalies, but which may also be due to ammoniacal decomposition, those constituents of the urine which are held in solution merely in consequence of the presence of acid sodium phosphate are also thrown down. In that case the sediment consists essentially of calcium, magnesium, and ammonium salts. Crystals of ammonio-magnesium phosphate, it is true, may also be observed in

alkaline urines of the first variety, but they are then almost always due to an increased elimination of ammonia, and hence are rarely observed under physiological conditions.

Normally calcium is found only in combination with phosphoric acid and carbonic acid. Of the three possible calcium salts of phosphoric acid—*i. e.*, $Ca_3(PO_4)_2$, $CaHPO_4$, and $Ca(H_2PO_4)_2$—only the first two are found in an alkaline urine, but they may also be observed in specimens which are either neutral or but faintly acid. The acid calcium phosphate, $Ca(H_2PO_4)_2$, is seen but rarely in sediments, and its occurrence always presupposes the existence of a high degree of acidity; it is precipitated together with uric acid and under similar conditions. Calcium carbonate, $CaCO_3$, is seen only in neutral or alkaline urines. As soon as ammoniacal fermentation has begun, ammonium salts are, of course, formed, viz., ammonium urate and ammonio-magnesium phosphate.

The following table shows the various mineral constituents usually observed in sediments, the reaction of the urine being in every case the all-important factor:

Reaction acid:
- Uric acid.
- Sodium urate.
- Calcium oxalate.
- Primary calcium phosphate.
- Ammonio-magnesium phosphate.

Reaction alkaline (referable to fixed alkalies):
- Secondary calcium phosphate.
- Tricalcium phosphate.
- Calcium carbonate.
- Ammonio-magnesium phosphate.

Reaction alkaline (referable to ammonia):
- Ammonium urate.
- Ammonio-magnesium phosphate.
- Tricalcium phosphate.
- Calcium carbonate.

In pathological conditions still other unorganized substances may be observed, viz., cystin, xanthin, hippuric acid, indigo, urorubin, bilirubin, hæmatoidin, magnesium phosphate, calcium sulphate, cholesterin, leucin, tyrosin, fats, soaps of magnesium and calcium, etc. Of these, cystin, xanthin, hippuric acid, tyrosin, calcium sulphate, bilirubin, hæmatoidin, magnesium phosphate, leucin, and the soaps of magnesium and calcium occur principally in acid urines, while indigo, urorubin, and cholesterin are usually only found in alkaline specimens. Before considering these various constituents in detail, a few words regarding sediments in general and the method to be followed in their microscopical examination may not be out of place.

An idea of the nature of a deposit may often be formed by simple inspection, especially if the reaction of the urine is known.

A crystalline sediment, presenting a brick-red color and appearing to the naked eye like cayenne pepper, is usually referable to uric acid. On the other hand, a deep-red amorphous deposit occurring in an acid urine consists essentially of urates, the color in this case, as in the former, being due to uroerythrin. Further proof is hardly required. Should doubt be felt, however, it will only be necessary to heat the urine, when the deposit will dissolve. A white flocculent sediment in an alkaline urine is usually referable to a mixture of phosphates and carbonates, and will dissolve without difficulty upon the addition of acetic acid, but remains unaffected by heat.

A sediment consisting of pus, and occurring in alkaline urines, is frequently mistaken for a phosphatic deposit by the beginner. Aside from a microscopical examination, this question may be settled by the addition of a small piece of caustic soda and stirring, when in the presence of pus the liquid becomes mucilaginous and ropy. If much pus is present, a tough, jelly-like mass will be formed, which escapes from the vessel *en masse* when the urine is poured out. Such a sediment, moreover, does not disappear upon the addition of an acid, and is rendered still more dense upon the application of heat.

Blood when present beyond traces may also be recognized.

As a general rule, the non-organized elements of a sediment are of little clinical interest.

Students are frequently in the habit of diagnosing an excessive, normal, or subnormal elimination of one or another urinary constituent from the result of a microscopical examination. This is unwarrantable, and it should always be remembered that no conclusions whatsoever can be drawn in this manner as to the amount actually eliminated. Nothing would be more erroneous than to infer an excessive excretion, not to speak of an excessive production, of uric acid or of oxalic acid from the fact that crystals of these substances are seen in large numbers under the microscope. Again and again cases are observed in which an excessive elimination of uric acid, oxalic acid, or phosphates is diagnosed by mere inspection, and in which a careful chemical analysis shows not only no increase, but even a diminution of the normal quantity.

A urine which is turbid when passed may be examined microscopically at once. As a rule, however, it is necessary to wait until a sediment has formed. To this end, the urine should be kept in a clean and well-stoppered bottle. A small amount of chloroform is added if necessary, and will preserve the specimen almost indefinitely. A few drops of the sediment are then removed by means of a *clean* pipette, carried down to the sediment, with the

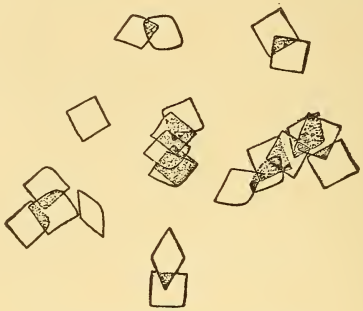
distal end tightly closed by the finger, care being taken not to allow the urine to *rush* into the tube by suddenly releasing the pressure, but withdrawing an amount just sufficient for an examination. This is then spread over a *clean slide* that has been moistened by the breath, when the specimen may be examined at once. *Covering the specimen with a slip is not only unnecessary, but even undesirable. A low power of the microscope should always be employed, and the high power only used to study details of structure.*

If a centrifugal machine is available, it is, of course, not necessary to let the urine stand until a sediment has formed. An amount sufficient for a microscopical examination can then be obtained in a few minutes.

Non-organized Sediments.

Sediments occurring in Acid Urines.—Uric Acid.—The form which uric acid crystals may present in a deposit varies greatly, the most common being the so-called whetstone-form shown in Fig. 107.

Fig. 117.



Colorless crystals of uric acid.

The crystals may occur singly or arranged in groups. Accidental impurities, such as threads or hairs, are at times covered with such crystals, forming long cylinders. Very frequently uric acid crystallizes in the form of large rosettes composed of drawn-out whetstone-crystals, presenting a deep-red color, referable to uroerythrin, when they are often visible to the naked eye, and form the well-known *brick-dust sediment.* While it is generally stated that uric acid crystals can always be recognized by their color, which may vary from a light yellow to a dark brown, this is, in my experience, not the case. I have often seen uric acid sediments in which the crystals formed small rhombic plates with rounded edges, and were absolutely devoid of coloring-matter, so far as a microscopical examination could show (Fig. 117). Uric acid "dumb-bells" are

also at times observed, and may be mistaken for calcium oxalate. Hexagonal plates of uric acid have been similarly confounded with cystin.

A uric acid sediment may be observed in cases in which an increased excretion of uric acid occurs; but it should be remembered that, as a rule, it is not permissible to infer an increased production or elimination from the presence of an abundant deposit of this substance alone. Brick-dust sediments are frequently observed during cold weather; but it would be erroneous to infer an increased elimination from such an occurrence, as the phenomenon is owing to the fact that uric acid is less soluble in cold than in warm water. During the summer months, for the same reason, a deposit of uric acid is less frequently observed, although an increased amount may nevertheless be present, being held in solution owing to the higher temperature. The more concentrated the urine and the more uric acid it contains, the more readily will such a deposit form. It is hence noted after profuse perspiration, following severe muscular exercise, in acute rheumatism with copious diaphoresis, in acute gastritis and enteritis associated with copious vomiting or diarrhœa, during the crisis of pneumonia (particularly if accompanied by much sweating), etc. In all these conditions, however, an increased elimination of uric acid does not necessarily take place, the all-important factors being the reaction of the urine, its degree of concentration, and the surrounding temperature.

Should formed concretions of uric acid—*i. e.*, uric acid gravel—be found in the urine, a direct indication is afforded to diminish the acidity of the urine and to increase the amount of water, so as to guard against the formation of renal or vesical calculus.

Chemically, the nature of a uric acid sediment may be recognized by the fact that the crystals dissolve upon the addition of sodium hydrate, and reappear in the rhombic form upon acidifying with hydrochloric acid. When heated with dilute nitric acid the beautiful red color of ammonium purpurate is obtained upon the subsequent addition of ammonia (murexid test), as described elsewhere (see page 449).

Amorphous Urates.—Sodium and potassium urate frequently, and especially in fevers, form sediments of such density that upon microscopical examination it is almost impossible to discern anything but innumerable amorphous granules scattered over the entire field and obscuring all other elements that may be present. Cells or casts will frequently be seen studded with these granules. In such cases it is best to heat the urine to a temperature of 50° C., and to filter it as rapidly as possible while hot, the contents of the filter being subsequently used for a microscopical examination.

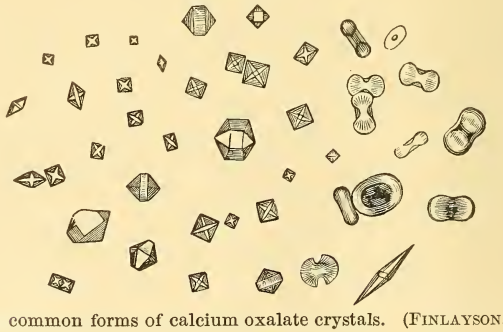
Urate sediments are always colored, the tint varying from a dirty brown to a bright salmon-red, owing to the presence of uroerythrin.

Difficulties can hence never arise in determining the nature of the sediment, as a colored deposit appearing in an acid urine which dissolves upon the application of heat cannot be due to anything but urates. If a drop of the sediment, moreover, is treated upon a slide with a drop of hydrochloric acid, characteristic whetstone-crystals of uric acid separate out, but the greater portion appears in the form of rhombic platelets.

Calcium Oxalate.—This substance generally appears in urinary sediments in the form of colorless, highly refractive octahedra (Fig. 118), which vary greatly in size; some appear as mere specks under even a comparatively high power, while others may attain the dimensions of a large leucocyte. Frequently one axis is longer than the other. From the fact that their diagonal planes are highly refractive, apparently dividing the superficial plane into four triangles, they have been compared to envelopes, and it is this envelope-form of the crystals which is especially characteristic. In the same specimen of urine so-called dumb-bell forms may be seen, which appear to be made up of two bundles of needle-like crystals united in the form of the figure 8. These, according to Beale, originate in the uriniferous tubules, and are frequently found adherent to or imbedded in tube-casts. Other forms may also be seen, and are shown in the accompanying figure.

While the envelope crystals are highly characteristic and can hardly be mistaken for any other substance, the student may at times confound them with crystals of ammonio-magnesium phosphate. This error may be avoided if it is remembered that the calcium oxalate crystals are usually not so large as those of the magnesium salt, and that the latter dissolve upon the addition of acetic acid, in which calcium oxalate is insoluble. The distinction from uric acid, if we are dealing with the dumb-bell form, cannot always be made by

FIG. 118.



Less common forms of calcium oxalate crystals. (FINLAYSON.)

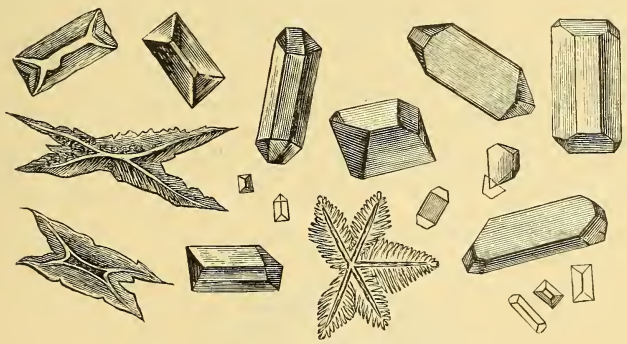
mere inspection. A drop of caustic soda should be added, which will dissolve the crystals if these are uric acid, while calcium oxalate remains unchanged.

It has been pointed out that under strictly normal conditions a few isolated crystals of calcium oxalate may be found in the primitive nubecula, so that their presence in urinary sediments cannot be regarded as pathological. After the ingestion of certain vegetables and fruits, notably rhubarb, garlic, asparagus, and oranges, or following the continued administration of sodium bicarbonate or the salts of vegetable acids, calcium oxalate crystals may be observed in large numbers; so also in certain diseases, such as diabetes mellitus, catarrhal jaundice, phthisis, emphysema, etc.

As in the case of uric acid, no inference as to the quantity eliminated can be drawn from a microscopical examination of the sediment. The frequent occurrence of abundant sediments of this substance may, however, generally be regarded as abnormal, providing that such an occurrence cannot be explained by the nature of the diet. It is very suggestive to note the frequency with which such sediments are observed in cases of neurasthenia, associated with a mild degree of albuminuria, as also in various digestive neuroses. Finally, as with uric acid, the possibility of the formation of renal calculi should be borne in mind whenever abundant sediments of calcium oxalate are encountered upon frequent examination.

Ammonio-magnesium phosphate, usually spoken of as triple phosphate, crystallizes in large prismatic crystals of the rhombic system;

FIG. 119.



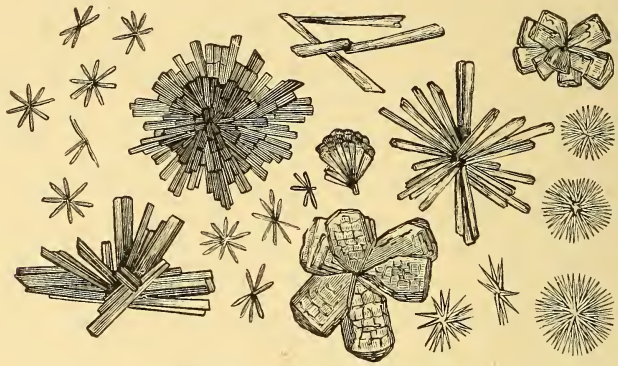
Various forms of triple phosphates. (FINLAYSON.)

it is most abundantly observed in alkaline urines, but may also occur in feebly acid specimens. Of the various forms which may occur, that resembling the lid of a German coffin is the most characteristic (Fig. 119). At times these crystals attain considerable size; very small specimens, however, also occur which may be mistaken for oxalate of calcium, but from these they are readily distinguished by the ease with which they dissolve in acetic acid, as has been pointed out.

Here, as elsewhere, it should be remembered that no conclusions

as to the amount actually eliminated can be drawn from a microscopical examination, and the diagnosis "phosphaturia" should be based only upon the results of a quantitative analysis.

Fig. 120.

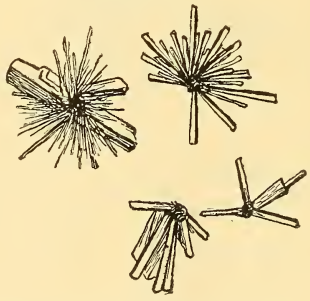


Crystalline phosphates. (Finlayson.)

The continued elimination of a turbid urine, the turbidity of which is referable to phosphates, is notably observed in neurasthenic individuals with a predominance of cerebral symptoms. Very curiously, the phosphaturia is not influenced by diet.

Monocalcium phosphate crystals are rarely seen, and only in specimens presenting a highly acid reaction, when uric acid crystals are also frequently observed in large numbers. I have seen only a few cases of this kind, occurring in patients the subjects of functional albuminuria. The urine was highly acid, in one case of a specific gravity of 1.036, and on standing deposited a sediment which consisted largely of monocalcium phosphate crystals (Fig. 121), with a considerable number of uric acid crystals, from which they are

Fig. 121.



Monocalcium phosphate crystals.

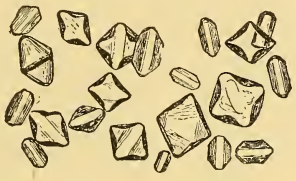
readily distinguished by the absence of pigment and their solubility in acetic acid.

Neutral Calcium Phosphate.—These crystals may be found in alkaline, neutral, and feebly acid urines. They are at times of large size, but more commonly acicular, occurring either singly or united in a star-like manner (Fig. 120). They are colorless, readily soluble in acetic acid, and insoluble in warm water, so that they can be easily distinguished from uric acid.

Basic magnesium phosphate crystals occurring in the form of large, highly refractive plates (Fig. 122), are at times seen in alkaline, neutral, or faintly acid and highly concentrated urines. They are readily recognized by treating a drop of the sediment upon a slide with a drop of ammonium carbonate solution (1 : 4), when the crystals become opaque and their edges assume an eroded aspect. In acetic acid they dissolve with ease and may then be reprecipitated by means of sodium carbonate.[1]

Hippuric acid crystals have been observed, although rarely, in urinary sediments, in acute febrile diseases, diabetes, and chorea; while their occurrence following the ingestion of large amounts of prunes, mulberries, blueberries, or the administration of benzoic acid and salicylic acid, is more common.

Fig. 122.



Basic magnesium phosphate crystals. (v. Jaksch.)

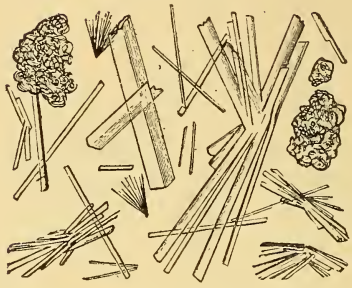
Hippuric acid occurs in the form of fine needles or rhombic prisms and columns, the ends of which terminate in two or four planes, at times resembling the crystals of ammonio-magnesium phosphate and of uric acid. From the former they may be readily distinguished by their insolubility in hydrochloric acid, and from the latter by the fact that they do not give the murexid reaction when treated with nitric acid and ammonia (see page 449). In the case of urines rich in hippuric acid in which the substance does not appear in the sediment, it is well to add a small amount of hydrochloric acid, when the crystals will gradually separate out. Their presence does not appear to possess any clinical significance.

Calcium sulphate, in the form of long colorless needles or elongated prismatic tablets (Fig. 123), has been observed in urinary sediments in only two cases. In both the urine, especially on standing, deposited a milky-looking sediment, the reaction being

[1] Stein, Arch. f. klin. Med., 1876, vol. xviii. p. 207.

strongly acid. It may be recognized by its insolubility in acids and ammonia.[1]

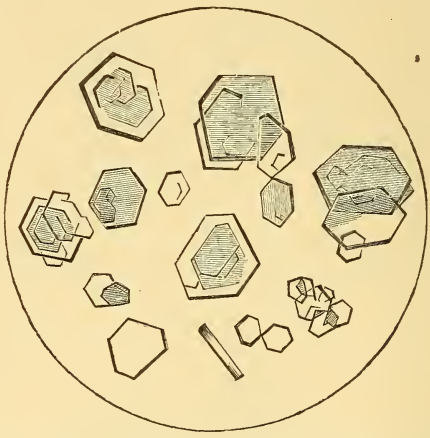
Fig. 123.



Calcium sulphate crystals. (v. Jaksch.)

Cystin ($C_6H_{12}O_4S_2$) is rarely seen in urinary sediments. It occurs in the form of colorless hexagonal platelets, which are very characteristic (Fig. 124). The crystals are soluble in ammonia and hydrochloric acid, and insoluble in acetic acid, water, alcohol, and ether.

Fig. 124.



Crystals of cystin spontaneously voided with urine. (Roberts.)

They can thus be readily distinguished from certain forms of uric acid, with which they might possibly be confounded at first sight. When heated upon platinum foil they burn with a bluish-green flame without melting.

Cystin-containing urines may be of normal appearance, but they often present a peculiar greenish-yellow color. Their reaction is mostly neutral or alkaline. Upon exposure to the air a marked odor

[1] v. Jaksch, Zeit. f. klin. Med., 1892, vol. xxii. p. 554.

of hydrogen sulphide develops, owing to decomposition of the cystin; but at times urines are met with in which a distinct odor of hydrogen sulphide is noticeable, although crystals of cystin are not seen in the sediment. It may then be demonstrated by strongly acidifying the urine with acetic acid or by allowing it to undergo ammoniacal decomposition. In either case cystin crystals will separate out on standing. It should be remembered, however, that not all urines in which hydrogen sulphide is formed contain cystin (see Hydrothionuria).

The amount of cystin which may be found in urinary sediments is variable. Sometimes a few centigrammes only are obtained, while at others from 0.5 to 1 gramme may be recovered. As is the case with the other non-organized constituents of sediments, however, the amount deposited does not necessarily indicate the total amount present. Where a quantitative estimation of cystin is to be made, it is best to filter off that which is deposited and to estimate the amount of neutral sulphur in the filtered urine. An increase beyond the normal may be referred to the cystin remaining in solution (see Neutral Sulphur).

Clinical interest in connection with cystinuria centres in the frequent association of cystin sediments with cystin gravel or calculi; but it is curious to note that the cystinuria, notwithstanding the removal of the calculus, may persist for years without giving rise to symptoms denoting the existence of a pathological process.

Very remarkable is the not uncommon occurrence of cystinuria in families. Cases of transient cystinuria likewise occur, and it is hence scarcely admissible to speak of a "cured" cystinuria when the condition disappears under treatment.

Of the origin of the condition little is known. It has been supposed that the appearance of cystin in the urine is in some manner connected with the formation of certain diamins in the intestinal canal. I have pointed out, however, that in all probability the formation of cystin and diamins takes place in the tissues of the body, and that the appearance of both is the expression of a definite metabolic anomaly rather than of a specific infection (see page 583).

LITERATURE.—C. E. Simon, "Cystinuria and its Relation to Diaminuria," Am. Jour. Med. Sci., 1900, vol. cxix. p. 39. See also the literature on page 584.

Leucin and **tyrosin** belong to the group of amido-acids, and are represented by the formulæ $C_6H_{13}NO_2$ and $C_9H_{11}O_3$. They are never found in urinary sediments under normal conditions, while traces of both substances may be present in solution. Larger amounts are notably found in acute yellow atrophy, of which disease their presence in sediments is almost pathognomonic. In acute phosphorus poisoning leucin and tyrosin are usually not found. The fact that urea may be altogether absent from the urine in acute yellow atrophy or present in greatly diminished amount has been previously referred

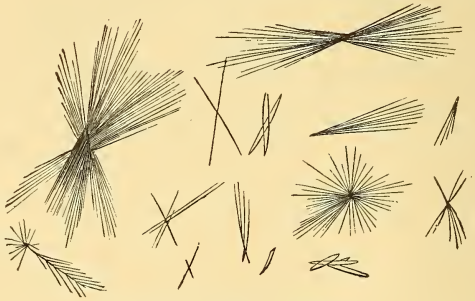
to (see Urea, page 421), and the elimination of leucin and tyrosin in its stead, as it were, has been regarded not only as indicating the probable origin of urea from amido-acids, but also the formation of urea, to a large extent at least, in the liver. The albuminous origin of these substances has also been noted (see Urea).

Traces of leucin and tyrosin are said to be constantly present in cases of cirrhosis and carcinoma of the liver, in cholelithiasis, catarrhal jaundice, Weil's disease, nephritis, cystitis, gout, bronchitis, tuberculosis, typhoid fever, hysteria, erysipelas, glucosuria, etc. In connection with cystinuria, the elimination of tyrosin has also been observed, but in two cases which I examined in this direction I obtained negative results. In diabetic urines both are supposedly absent.

As leucin is hardly ever found in the sediment, and tyrosin only when present in large quantities, the urine in every case should first be concentrated upon a water-bath and examined on cooling. At times, however, when these substances are present in only very small quantities, this procedure may not lead to the desired end, and in doubtful cases the following method should be employed :

The total amount of urine voided in twenty-four hours is precipitated with basic lead acetate and filtered, when the filtrate, from which the excess of lead has been removed by means of hydrogen sulphide, is evaporated to as small a volume as possible, and is set aside for crystallization. The residue thus obtained is then examined with the microscope; if crystals are detected which answer the description of tyrosin and leucin, they should be subjected to further chemical tests.

Fig. 125.



Tyrosin crystals. (Charles.)

Ulrich advises to evaporate the urine to dryness and to heat the residue gently while the vessel is covered with a plate of glass or a funnel. The tyrosin is then said to sublime, and is deposited on the cool glass in crystalline form, the crystals giving the characteristic reactions.

Tyrosin crystallizes in the form of very fine needles (Fig. 125),

which are usually grouped in sheaves or bundles crossing each other at various angles. They are insoluble in acetic acid, but soluble in ammonia and hydrochloric acid.

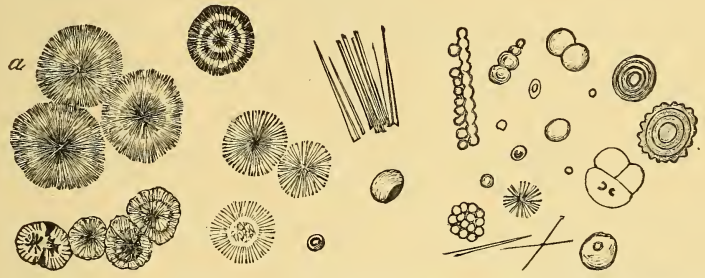
Leucin (Fig. 126) occurs in the form of spherules of variable size, which closely resemble globules of fat, but may be distinguished from these by their insolubility in ether. In the urine they present a more or less pronounced brownish color, and upon close examination concentric striations as well as very fine radiating lines can at times be made out, which are especially characteristic.

If crystals resembling tyrosin and leucin are found, the following tests should be made :

Tests for Tyrosin.—The sediment is filtered off, washed with water and dissolved in ammonia to which a little ammonium carbonate has been added. The solution is allowed to evaporate, when the tyrosin remains behind.

Piria's Test.[1]—A bit of the tyrosin is moistened on a watch-crystal with a few drops of concentrated sulphuric acid, covered, and set aside for half an hour. It is then diluted with water, heated, and while hot saturated with calcium carbonate and the solution filtered. The filtrate is colorless, but when heated with a few drops of a very dilute solution of ferric chloride, which must be free from hydrochloric acid, it assumes a violet tint (v. Jaksch).

Fig. 126.



Crystals of leucin (different forms). (Crystals of kreatinin-zinc chloride resemble the leucin crystals depicted at *a*.) The crystals figured to the right consist of comparatively impure leucin. (Charles.)

Hoffmann's Test.[2]—A small amount of tyrosin is dissolved in hot water and treated, while hot, with mercuric nitrate and potassium nitrite. The solution assumes a beautiful dark-red color and yields a voluminous red precipitate.

Tests for Leucin.—*Scherer's Test.*[3]—To test for leucin, this is separated from tyrosin by the addition of a little alcohol (see below). The alcohol is allowed to evaporate, and a portion of the residue

[1] Piria, Liebig's Annal., 1852, vol. lxxxii. p. 251.
[2] Hoffmann, Ibid., 1857, vol. lxxxvii. p. 124.
[3] Scherer, Jour. f. prak. Chem., 1887, vol. lxxix. p. 410.

treated upon platinum foil with nitric acid, when a colorless residue is obtained which, upon the application of heat and the addition of a few drops of a solution of sodium hydrate, forms a droplet of an oily fluid which does not adhere to the platinum.

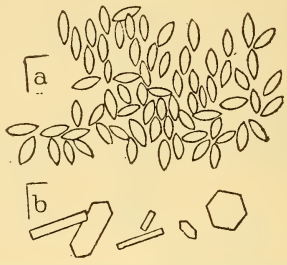
Hofmeister's Test.[4]—A small amount of leucin dissolved in water causes a deposit of metallic mercury when heated with mercurous nitrate.

In order to separate the leucin from the tyrosin, the sediment is treated with a small amount of alcohol, in which leucin is more readily soluble than tyrosin.

LITERATURE.—Frerichs, Wien. med. Woch., 1854, vol. iv. p. 465. Schultzen u. Riess, Charité Annal., vol. xv. Pouchet, Maly's Jahresber., 1880, vol. x. p. 248. Irsai, Ibid., 1885, vol. xiv. p. 451. Prus, Ibid., 1888, vol. xvii. p. 345. Fränkel, Berlin. klin. Woch., 1878, vol. xv. p. 265.

Xanthin crystals (Fig. 127) are very rarely observed in urinary sediments, and, so far as I have been able to ascertain, the case observed by Bence Jones[1] is the only one on record. Care should be had not to confound certain forms of uric acid with xanthin, and I well remember an instance in which crystals were observed

FIG. 127.



a, Crystals of xanthin (SALKOWSKI); *b*, Crystals of cystin (ROBIN).

identical in appearance with those here pictured, but which upon chemical examination proved to be uric acid. *The necessity of disregarding the statement generally made that uric acid crystals found in urinary sediments are invariably colored cannot be insisted upon too strongly.* It has been stated elsewhere that colorless uric acid crystals may be encountered, and in the case just cited such were observed.

Clinically, xanthin sediments are of interest only in so far as this substance may give rise to the formation of calculi; in the case observed by Bence Jones attacks of renal colic had occurred several years previously.

Soaps of Lime and Magnesia.—v. Jaksch has pointed out that in various diseases crystals may be found which "closely" resemble

[1] Hofmeister, Liebig's Annal., 1877, vol. cxxxix. p. 6.
[2] Bence Jones, Chem. Centralbl., 1868, vol. xiii.

tyrosin in appearance, and pictures such crystals (Fig. 128), which from their behavior toward reagents he is inclined to regard as calcium and magnesium salts of certain higher fatty acids.

FIG. 128.

Lime and magnesium soaps. (V. JAKSCH.)

Should doubt arise, the question may be readily decided by a chemical examination (see tests for tyrosin and fatty acids).

Bilirubin crystals in the form of yellow or ruby-red rhombic plates or needles, as well as amorphous granules, have been seen in the urine in rare cases, but are of no special interest. They are easily soluble in alkalies and chloroform, but not in ether. When treated upon a slide with a drop of nitric acid a green ring will be seen to form around them (Gmelin's reaction).[1] Such crystals have been found in icteric urine and in a case of pyelonephritis.

Hæmatoidin crystals are likewise only rarely seen. They cannot be distinguished from bilirubin, with which, indeed, they are supposedly identical.[2] They may be found either free or imbedded within cells or tube-casts, in cases of scarlatinal nephritis, the nephritis of pregnancy, in granular atrophy, amyloid degeneration of the kidneys, and in carcinoma of the bladder, of which latter condition they have been regarded by some as pathognomonic.

Fat.—When small, strongly refractive globules of fat, which may be readily recognized by their solubility in ether, are observed either floating on the urine or held in suspension, it is necessary to ascertain first of all whether such fat may not be present accidentally, owing to the use of a bottle or vessel not absolutely clean, or previous catheterization, etc. The diagnosis *lipuria* should only be made when all possible precautions have been taken to insure

[1] Kussmaul, Würzburger med. Zeit., 1863, vol. iv. p. 64. Ebstein, Arch. f. klin. Med., 1879, vol. xiii. p. 115.

[2] Hoppe-Seyler u. Thierfelder, Handb. d. physiol. u. path., chem. Analyse.

against the *accidental* presence of this substance. Every physician who has frequent occasion to examine urines has undoubtedly met with instances in which fat-globules were found, and in which careful inquiry showed that these were accidentally present. True lipuria—*i. e.*, an elimination of fat usually in the form of droplets floating on the urine—has been noted in various cachectic conditions, in cases of heart-disease, affections of the pancreas and liver, in gangrene and pyæmia, in diseases of the bones, especially following fractures, in diseases of the joints, etc. Fat has also been observed in the urine following the ingestion of large amounts of cod-liver oil and inunctions with fats and oils.

In fatty degeneration of the kidneys, in Bright's disease, phosphorus poisoning, etc., droplets of fat may be seen in the epithelial cells and tube-casts. This, however, does not constitute lipuria. The nature of the droplets may be recognized by their solubility in ether, benzol, chloroform, carbon disulphide, xylol, etc., and by the fact that they are colored black when treated with a 0.5 to 1 per cent. solution of osmic acid, and red when a drop of tincture of alcanna is added to the specimen. A very convenient method of demonstrating the presence of fat is also the following: a few cubic centimeters of the urine are mixed with an equal volume of 96 per cent. alcohol and a concentrated solution of Sudan III. in 96 per cent. alcohol. The sediment which collects is then examined under the microscope; the excess of stain is removed by allowing a few drops of 60 or 70 per cent. alcohol to run under the cover-slip and removing it with filter-paper placed at the edge of the preparation. The fat-droplets are thus colored an intense scarlet red, while granules of albuminous origin are unstained. Free fat can, of course, be demonstrated in the same manner.

The largest amounts of fat are observed in chyluria, a condition which is usually due to the presence of a specific parasite in the blood, viz., the Filaria sanguinis hominis, or more rarely the Distoma hæmatobium, which have been described in the chapter on the Blood (see also Chyluria).

Sediments occurring in Alkaline Urines.—Basic Phosphate of Calcium and Magnesium.—The most common sediments observed in alkaline urines consist of amorphous phosphates of calcium and magnesium. They are usually as abundant as the urate sediments which have been described, but may be readily distinguished from these by the fact that they do not dissolve upon the application of heat, but readily disappear upon the addition of acetic acid, and are never colored. In this manner it is also easy to distinguish such a sediment from one due to pus, with which it might possibly be confounded at first sight. Upon microscopical examination a drop of the sediment will be seen to contain innumerable transparent granules

scattered over the entire field, and closely resembling those of urate of sodium and potassium.

Phosphatic sediments are observed, as mentioned elsewhere, whenever the reaction of the urine is alkaline, whether this be owing to the presence of fixed alkali or to ammoniacal fermentation.

Ammonium urate is observed only in urines which are undergoing ammoniacal decomposition. Its presence should always call for a careful investigation in order to ascertain whether this has taken place after the urine has been voided or before (see Reaction).

The salt occurs in the form of colored spherical bodies of variable size, which are sometimes composed of delicate needles, while at others they are amorphous, but may be beset with prismatic spicules. They are not easily mistaken for any other substance which may be present in urinary sediments (Fig. 129). Ammonium urate is characterized, moreover, by its solubility in acetic and hydrochloric acids, and by the subsequent separation of rhombic crystals of uric acid.

Magnesium phosphate has been described above (see page 593).

Ammonio-magnesium Phosphate.—While the well-known coffin-lid crystals are commonly seen in feebly acid urines, as pointed out, ammonio-magnesium phosphate presents a great variety of forms in alkaline urines, and especially in specimens undergoing ammoniacal decomposition (see Fig. 120).

FIG. 129.

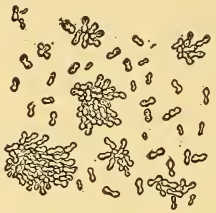
Ammonium urate crystals.

Calcium carbonate frequently occurs in alkaline urines, and appears under the microscope in the form of minute granules, occurring singly or arranged in masses; dumb-bell forms are also seen (Fig. 130). They may be recognized by the fact that they readily dissolve in acetic acid with the evolution of gas.

Indigo in the form of delicate blue needles, arranged in a stellate manner or in plates, visible only with the microscope, is rarely seen.

In an amorphous condition, however, it may be met with in almost every decomposed urine, occurring in the form of small granules,

Fig. 130.



Calcium carbonate crystals.

and frequently staining the morphological elements that may be present a distinct blue. Sediments presenting a bluish-black color were noted in the time of Hippocrates already, and have been described since by numerous observers, but the nature of the coloring-matter has only been determined within the last fifty years. Clinically, the occurrence of indigo in the urine is of interest, as renal calculi have been observed which consisted almost entirely of this substance. But little is known of the causes which give rise to its appearance in the urine, but there can be no doubt that its occurrence is referable to the action of certain micro-organisms upon urinary indican (see page 559).[1]

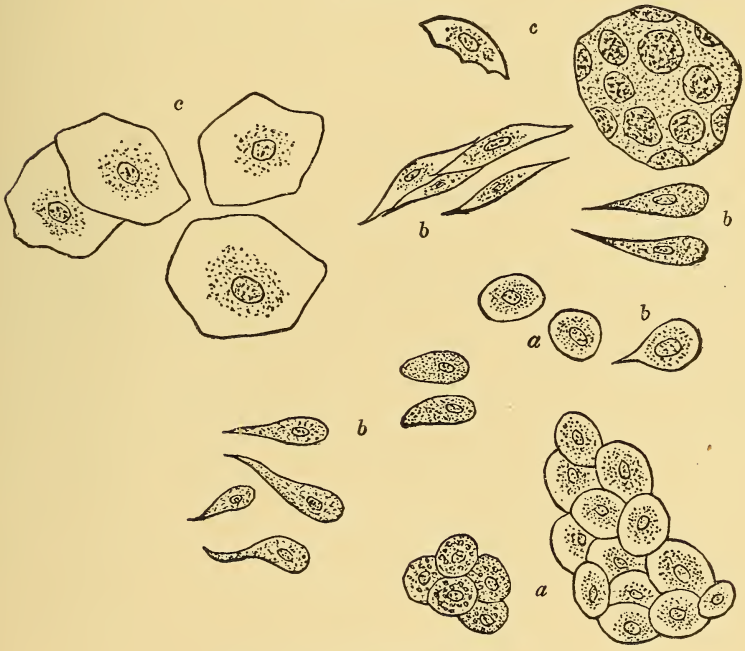
Organized Constituents of Urinary Sediments.

Epithelial Cells (Fig. 131).—Bearing in mind the fact that desquamative processes are constantly going on in the epithelial lining of the various cavities and channels of the body, one should expect to find in every urine representatives of the different forms of epithelium occurring in the urinary organs, from the Malpighian tufts down to the meatus urinarius. To a certain extent this actually happens, and cells apparently derived from the meatus, the urethra, bladder, ureters, and pelvis of the kidneys may be met with in almost every specimen, although it may at times be difficult to refer to their origin the individual cells observed. Bizzozero even claims that it is impossible to distinguish between the cells of the bladder and those of the meatus and renal pelvis, while as a class they may readily be differentiated in most cases from the cells of the urethra, the ureters, the prepuce of the male, and the vulva and vagina of the female. Cells from the uriniferous tubules of the kidneys are seldom seen in normal urines, and when they do occur it is impossible to determine their exact origin—*i. e.*, the particular

[1] v. Jaksch, Prag. med. Woch., 1892, vol. xvii. p. 602.

portion of the tubule from which they have been detached. Cells presenting the characteristic striated appearance seen in the irregular, and to a less evident degree in the convoluted, portions of the uriniferous tubules, are never observed in the urine. This fact, as well as the usual absence of true glandular cells, remains to be explained. It is not improbable that the absence of these cells may be referable to a less marked desquamation going on in those parts in which the mechanical injury to which the epithelium is subject must of necessity be far less severe than in the remaining portions of the urinary tract, and particularly in the bladder and urethra.

FIG. 131.



Epithelium from the urinary passages.
a, Round cells; *b*, conical and caudate cells; *c*, flat cells.

As stated elsewhere, the number of epithelial cells occurring in urinary sediments under physiological conditions is small, and the presence of large numbers may hence always be regarded as abnormal, and indicating the existence of a circulatory or inflammatory disturbance affecting some portion of the urinary tract.

Were it possible in every case to determine the exact origin of the cells, it is evident that information of great value would thus be obtained. Unfortunately, this is not always possible, as the form of the cells is dependent to a certain extent upon the reaction of the urine, an alkaline or neutral reaction causing the cells

to swell and to appear larger and rounder than is the case in acid urines. As has been mentioned, the cellular type is practically the same, moreover, in the bladder, ureters, and pelvis of the kidneys.

Definite conclusions should hence be drawn only exceptionally from a microscopical examination alone, but there can be no doubt that in conjunction with other factors and the clinical history the demonstration of a normal or increased number of epithelial cells may frequently be of decided value in a differential diagnosis, and taking these factors into consideration it may even be possible to localize the seat of the lesion. If attention is directed to the structure of the individual cell—and this holds good more especially for the cells derived from the uriniferous tubules—an idea may at times even be formed of the character of the lesion (see below).

Ultzmann recognizes three forms of epithelial cells which may be found in urinary sediments, viz.:

1. Round cells.
2. Conical and caudate cells.
3. Flat cells.

Round cells are usually derived from the uriniferous tubules and the deeper layers of the mucous membrane of the pelvis of the kidneys. In the urine they present a more or less rounded form and are provided with a distinct nucleus; they are not much larger than pus-corpuscles. From the latter they are distinguished by the presence of a well-defined nucleus, which in pus-cells becomes distinct only upon the addition of acetic acid, and is, moreover, polymorphous. Whenever such cells are found adhering to urinary casts, which may at times consist entirely of these structures, it is clear that they represent the glandular elements proper of the kidneys. As similar cells are found in the male urethra, confusion may possibly arise. Should albumin, however, be present, the cells are probably of renal origin. The presence of such cells in large numbers together with pus, in the absence of tube-casts and albumin beyond traces, will usually indicate the existence of a simple pyelitis, particularly if round cells are found joined in a shingle-like manner. Should the pyelitis be associated with a nephritis, tube-casts and albumin in larger amounts will at the same time be present. In such cases it may be impossible to determine the origin of the cells, excepting of such that may adhere to casts. In simple circulatory disturbances affecting the renal parenchyma no special abnormalities can be discovered in the structure of the cells, while in cases of fatty degeneration of the kidneys they will be seen to contain fatty particles in greater or less abundance, so that it may be possible to determine the existence of degenerative processes which may be of inflammatory or non-inflammatory origin. The same may be said to hold good if the epithelial elements are markedly granular and occur in fragments.

Conical and **caudate cells** are mostly derived from the superficial layers of the pelvis of the kidneys, and are hence especially seen in cases of pyelitis. Similar cells are also found in the neck of the bladder, and may usually be distinguished from those of the pelvis by the greater length of their processes.

Flat cells may come from the ureters, the bladder, the prepuce of the male, and the vulva and vagina of the female. These cells present the usual characteristics of squamous epithelium, being large, polygonal in form, and provided with a well-defined nucleus; the extra-nuclear protoplasm is only slightly granular. Other more or less rounded forms are also seen, which are derived from the deeper layers of the mucosa, but may be distinguished from the small round cells of the kidneys proper. Irregular or conical cells, often provided with one or more protoplasmic processes, likewise come from the lower layer of the mucosa of the bladder and ureters.

While the cells of the bladder may thus be confounded with those of the ureters and vagina under the microscope, it is not likely that a vaginitis or vulvitis will be mistaken for a cystitis or a ureteritis. In doubtful cases specimens of urine should be procured by means of the catheter, care being taken to first thoroughly cleanse the vulva. The warped appearance so frequently seen in vaginal epithelial cells, and the fact that they often and indeed usually appear in masses, may further aid in the differential diagnosis.

It has been pointed out by Peyer that the presence of pavement-epithelial cells, together with mucus and leucocytes, in the urine of hysterical and anæmic girls may be regarded as indicating an irritable condition of the genitals, possibly in consequence of masturbation. Bearing in mind the moist and sensitive condition of the vulva of female masturbators, such a view is plausible.

A ureteritis, notwithstanding the fact that the ureteral cells closely resemble those of the bladder, may be inferred indirectly, the presence of squamous cells in abundance pointing to a cystitis, a small increase in their number to ureteritis. In conclusion, it should be stated that the so-called mucous corpuscles present in every urine are young vesical cells.

From what has been said, it is clear that, with due precautions and taking other factors into consideration, the discovery of epithelial cells in large numbers in urinary sediments may be of decided value in diagnosis.

LITERATURE.—Bizzozero, loc. cit. Eichhorst, Lehrbuch d. physikal. Untersuch. inn. Krankheit., 2d ed., p. 336, Braunschweig.

Leucocytes.—Leucocytes are encountered in only very small numbers in normal urines. A marked increase should, hence, always be regarded as indicating the existence of disease somewhere in the course of the urinary tract, excepting in females, where their presence

may be owing to an admixture of leucorrhœal discharge. In that case the source of the pus will generally be recognized by the simultaneous occurrence of pavement epithelial cells of the vaginal type in correspondingly large numbers. In doubtful cases the urine should always be obtained with the catheter, care being taken to thoroughly cleanse the vulva before the introduction of the instrument.

Occasionally the pus is derived from a neighboring abscess that has opened into the urinary passages.

The amount of pus which may be found in urines is most variable. On the one hand, deposits several centimeters in height are not uncommon, and closely resemble deposits of phosphates in appearance, for which they are indeed frequently mistaken; on the other hand, it may only be possible to discover the presence of pus by means of the microscope, which should be employed in every case.

The appearance of the pus-corpuscles likewise varies in different cases. In acid urines their form is usually well preserved, and in feebly alkaline and neutral specimens it may even be possible to observe amœboid movements when the slide is carefully warmed. In alkaline urines, however, they usually swell up and become opaque, so that it is impossible to discern a nucleus unless they are treated with acetic acid. At other times, and particularly when pus has remained long in the body, as where an abscess has burst into the urinary passages, it may be almost impossible to make out a nucleus, and in extreme instances nothing but a mass of granular and fatty detritus is left.

While with a certain amount of experience it is hardly likely that a sediment of pus will be mistaken for anything else, such as a deposit of phosphates, it should be remembered that if pus is exposed to the action of ammonia or an ammonium salt the pus-corpuscles become disintegrated. In such cases, as in cystitis, in which ammoniacal decomposition of the urine has taken place in the bladder, a deposit may be obtained which macroscopically resembles mucus, and in which pus-corpuscles may not even be demonstrable with the microscope. The sediment then escapes as a gelatinous, slippery mass when the urine is poured from one vessel into another. Recourse must then be had to certain chemical tests, as a pyuria might otherwise be overlooked. To this end, the following procedure, suggested by Vitali,[1] may be employed:

The urine, after having been acidified with acetic acid, is filtered, and the contents of the filter treated with a few drops of tincture of guaiacum which has been kept in the dark, when in the presence of pus the filter-paper is colored a deep blue. The reaction is supposedly due to the presence in the leucocytes of specific nucleoproteids.

[1] Vitali, Maly's Jahresber., 1890, vol. xviii. p. 326.

A solution of iodo- potassic iodide may be employed in less extreme instances. A drop of this solution is added to a drop of the sediment upon a slide, when the pus-corpuscles, owing to the presence of glycogen, are colored a dark mahogany-brown, while epithelial cells, with certain forms of which they might possibly be mistaken, assume a light color.

Donné's pus-test is based upon the fact that the transformation of pus into a gelatinous, mucus-like mass, observed in cases of cystitis, owing to the action of ammonium carbonate, may also be artificially produced by the addition of a small piece of caustic soda and stirring, when in the presence of pus in small amounts the liquid becomes mucilaginous and ropy, while a gelatinous mass is obtained if it is abundant.

From a clinical point of view it is most important to establish the source of the pus in every case of *pyuria*. This may at times be difficult, but the following data will be found of value in a differential diagnosis:

1. In diseases affecting the renal parenchyma the amount of pus, as a rule, is small, except where a large abscess located in the kidney structure proper has suddenly burst into the pelvis of the kidney.

In uncomplicated cases it is a comparatively easy matter to recognize the renal origin of the pus, as other constituents, such as renal epithelial cells, and especially tube-casts, are usually present at the same time, and, as was noted in the case of renal epithelial cells, leucocytes are frequently found adhering to the tube-casts, and at times apparently compose these entirely, when they are spoken of as *pus-casts* (see Casts). In nephritis, according to Bizzozero, the number of pus-corpuscles stands in a direct relation to the intensity and acute character of the morbid process, the greatest number being found in cases of acute nephritis, while in the chronic forms their number is usually insignificant. Whenever in the course of a chronic nephritis large numbers of pus-corpuscles appear, they may be regarded as indicating either an acute exacerbation of the disease or a complicating inflammation of some portion of the urinary tract. In such cases errors may be guarded against by carefully observing the number and character of the epithelial cells present at the same time, when it will often be found that what at first sight appears as an acute exacerbation of a chronic process, judging from the number of pus-corpuscles, is in reality a secondary pyelitis, ureteritis, or cystitis.

In cases of simple renal hyperæmia pus-corpuscles never occur in notable numbers.

2. In pyelitis the amount of pus eliminated may vary considerably, and at times even perfectly normal urine may be voided. This is probably owing to the fact that the ureter of the affected side, if the disease is unilateral, becomes obstructed temporarily, when sud-

denly large quantities may appear again. The diagnosis of pyelitis is often difficult, and should be based not only upon the condition of the urine, but upon the clinical symptoms as well. Very significant is the fact that the urine in pyelitis is usually acid, a point to be remembered in the differential diagnosis between this condition and cystitis, with which pyelitis is frequently confounded. A careful examination of the epithelial elements may also be of value, and should never be neglected. Bacteria in large numbers are generally present.

When pyelitis is associated with nephritis it may at times be almost impossible to determine the origin of the pus; but if the rule set forth above is remembered, that in chronic nephritis the number of leucocytes is always small, it is not likely that a pyelitis will be overlooked, particularly if the clinical symptoms are taken into consideration.

Matters may become still more complicated when a cystitis is accompanied by a pyelitis or a pyelonephritis. Catheterization of the ureters, which was first practised in the United States by the late Dr. James Brown, should then be resorted to, and it is highly desirable that this most valuable method of diagnosis should become common property as soon as possible. Fischl regards the presence of cylindrical masses composed of pus-corpuscles, formed in all probability in the papillary ducts, as highly characteristic of pyelitis.

3. A pyuria referable to ureteritis can hardly be diagnosed from the appearance of the urine, and in suspected cases catheterization of the ureters should be resorted to, which may possibly elicit information of value.

4. In mild cases of cystitis pus may be altogether absent, while in the more severe forms its presence is constant. In cystitis the largest amounts, referable to disease of the urinary organs, are observed, and are exceeded only in those rare conditions in which a neighboring abscess has suddenly opened into the urinary passages.

As the urine in cystitis is usually alkaline, and always so in the more severe forms, the alkalinity being due to ammoniacal fermentation, it may happen, owing to the disintegrating action of the ammonium carbonate upon the pus-corpuscles, that these may not even be demonstrable with the microscope, and that a gelatinous, mucoid sediment appears instead, which escapes from the vessel *en masse* when the urine is poured out. Vitali's test for pus (referred to on page 606) should be employed in such cases.

5. In urethritis pus may be present in the urine in considerable amounts. The source of the pus is recognized by the fact that a drop may be manually expressed from the urethra, particularly in the morning upon awaking. Mucoid gonorrhœal threads,—the "Tripperfäden" of the Germans,—which are largely composed of

pus-corpuscles, will almost always be detected in the urine in such cases (Fig. 140). In order to distinguish between a simple urethritis and a urethritis complicated with cystitis, the urine should be obtained in two portions and allowed to settle. In simple urethritis affecting the anterior portion of the urethra the first specimen is cloudy, while the second one is clear. If the urethritis, however, has extended to the neck of the bladder, in the absence of cystitis, the first portion will, of course, be cloudy, while the second may present a variable appearance, being clear at times and cloudy at others. This phenomenon is explained by the fact that a portion of the pus contained in the posterior portion of the urethra has found its way into the bladder. A cystitis may, however, be excluded by the acid reaction of the second specimen, and the fact that the latter is never so cloudy as the first. In cases of urethritis complicated with a purulent cystitis the second portion of the urine contains at least as much pus as the first, and usually more, owing to the fact that the pus (which is heavier than the urine) falls to the floor of the bladder, in which case also the last drops passed will often be found to be pure pus. The reaction of the urine, moreover, will then be generally alkaline.

6. A sudden elimination of large quantities of pus with a urine which up to that time has presented a normal or nearly normal appearance may almost always be referred to rupture of an abscess into the urinary passages. Exceptions to this rule have been noted in rare instances in which large amounts of pus suddenly appeared, the origin of which could not be demonstrated upon post-mortem investigation. Whether such a phenomenon, as v. Jaksch suggests, is dependent upon "unusual conditions favoring diapedesis" remains an open question.

Enumeration of the Pus-corpuscles in the Urine.—In order to determine the relation existing between the degree of pyuria and albuminuria, as well as to watch the progress of an individual case, an enumeration of the number of pus-corpuscles is at times necessary. To this end, a specimen of the urine is thoroughly shaken and the number of corpuscles contained in one cubic millimeter ascertained with the aid of the Thoma-Zeiss blood-counter. Dilution with a 3 per cent. solution of common salt is necessary when a preliminary examination has shown the presence of more than 40,000 corpuscles per cbmm. A dilution of five times is usually sufficient. In every case one hundred squares at least should be counted.

Some of the results which have thus been obtained are extremely interesting. In cases of mild cystitis 5000 pus-corpuscles are found on an average in the cubic millimeter; in cases of moderate severity from 10,000 to 20,000; while in severe cases 50,000 and even more may be seen. In one case of cystitis complicating carcinoma of the bladder Hottinger obtained 152,000 per cbmm. In the presence of

less than 50,000 a mere trace of albumin is found, and with 80,000–100,000 only 1 pro mille is referable to this source.[1]

Red Blood-corpuscles.—The presence of red blood-corpuscles in the urine, constituting the condition usually spoken of as *hæmaturia*, is observed only in pathological conditions, and is, in contradistinction to hæmoglobinuria (which see), a very common occurrence.

Urine containing blood-corpuscles in notable numbers presents a color which may vary from a bright red to a dark brown verging upon black. Upon standing, a sediment of a corresponding color is obtained in which distinct coagula of variable size are at times seen.

If the urine should contain only a small number of red corpuscles, however, no deviation from its normal appearance will be noted, and the diagnosis of hæmaturia can then only be made with the microscope, which should be employed in every case. The appearance of the red corpuscles varies greatly, being influenced especially by the length of time during which they have remained in the urine. In cases of hæmaturia of urethral or vesical origin it will be found that they have mostly retained their normal appearance fairly well, or have become crenated, when they may be recognized without difficulty. Other corpuscles, however, will probably also be seen which are no longer biconcave, but which have become spherical or shrunken and present an irregular outline. In cases, on the other hand, in which the corpuscles have remained in the urine for a longer time, as in hæmaturia of renal origin, the inexperienced will frequently be puzzled by the presence of bodies of the size of red corpuscles, or somewhat smaller, which are entirely devoid of coloring-matter, and appear as faint, transparent rings, often presenting a double contour, and in which no nucleus can be discovered. These formations are red blood-corpuscles from which the hæmoglobin has been dissolved. They are usually spoken of as *blood-shadows*. Chemical tests are rarely necessary, but may be employed if doubt should arise (see page 507).

Clinically it is, of course, all-important to determine the source of the blood. This may at times be accomplished without much difficulty by a urinary examination, but at other times it may almost be impossible, when the clinical symptoms and physical signs must be taken into consideration.

1. Hæmaturia of urethral origin, due to urethritis or traumatism incident to catheterization, for example, is a common event, and readily diagnosed, as in such cases blood either escapes of itself from the urethra or it may be squeezed out manually. The last portion of the urine voided, moreover, will always be found free from blood, unless it is referable to disease of the neck of the bladder,

[1] R. Wunderlich, Ueber d. Werth d. Zählung d. weissen Blutkörperchen im Harn, etc., Diss., Würzburg, 1885.

when the blood appears only toward the end of micturition, or at least more markedly then than in the beginning.

2. The diagnosis of vesical hæmaturia is not always easily made. It should be remembered, however, that the blood-corpuscles here present a normal appearance, as has been mentioned, unless ammoniacal decomposition is occurring in the bladder, in which case blood-shadows are seen in large numbers. The blood, moreover, is less intimately mixed with the urine than in cases of renal hæmaturia, so that the corpuscles rapidly settle after the urine has been passed. Blood-clots of an irregular form and considerable dimensions can only be of vesical origin. A careful examination for the presence of any other morphological constituents which may be observed in urinary sediments, when considered in conjunction with the clinical symptoms, will usually lead to a correct diagnosis so far as the seat of the hemorrhage is concerned. Hæmaturia of vesical origin may be due to numerous causes, among which may be mentioned diphtheritic cystitis, ulcers of the bladder caused by calculi and carcinoma, traumatism, the presence of parasites, and, more rarely, rupture of varicose veins in the bladder. In determining the cause of the hemorrhage in a given case more reliance should be placed upon the clinical history than upon the urinary examination.

3. In hæmaturia of ureteral origin characteristic blood-coagula, corresponding in diameter and form to the ureters, are occasionally seen. Their presence, however, does not necessarily indicate that the blood has come from the ureters; more frequently the hemorrhage will be found to be due to disease of the pelvis of the kidney.

4. The diagnosis of hemorrhage into the pelvis of the kidney must be based upon the clinical symptoms taken in conjunction with the results of a urinary examination. In nephrolithiasis only a small number of red corpuscles is usually found, which is important from the standpoint of differential diagnosis.

5. Hæmaturia of purely renal origin is of common occurrence, and may be due to numerous causes. In simple hyperæmic conditions of the organs and in acute nephritis the passage of smoky-looking urine containing blood-corpuscles, usually in large numbers, is thus a fairly constant symptom. In chronic nephritis the number of the red corpuscles may be taken to indicate the intensity of the morbid process. Hæmaturia may also be due to renal abscess, nephrophthisis, renal carcinoma, and, in rare instances, to aneurism and embolism of the renal artery, thrombosis of the renal vein, etc. In the malignant forms of the acute infectious diseases, such as small-pox, yellow fever, malaria, etc., in scurvy, hæmophilia, and purpura, in leukæmia, filariasis, and distomiasis, renal hæmaturia is common. It is also observed in cases of poisoning with turpentine, carbolic acid, cantharides, and has recently also been observed in several convalescents

from typhoid fever while under treatment with urotropin ; the hæmaturia ceased with the discontinuance of the drug.[1]

6. An idiopathic form of hæmaturia has also been described, in which hemorrhage from the kidneys occurs without apparent cause. To this form Senator applied the term "renal hæmophilia." I have seen three cases of this kind in which no lesion existed which could be made responsible for the hemorrhage. In all three the attacks of hæmaturia were invariably associated with anachlorhydria, while normal values were found between the attacks. Two of the patients were males, and undoubtedly neurasthenics. The third was a hysterical chlorotic female, in whom hæmatemesis, pulmonary hemorrhages, and melæna were also at times observed.

Hæmaturia of renal origin is usually recognized without much difficulty, as in such cases tube-casts bearing red blood-corpuscles, and at times apparently consisting of these altogether, as well as numbers of renal epithelial cells, will usually be found upon careful examination. The blood, moreover, is intimately mixed with the urine, and the individual corpuscles have mostly lost their hæmoglobin and appear as mere shadows. The clinical history should, of course, always be taken into consideration, especially in determining the primary cause of the hemorrhage.

Urine containing red blood-corpuscles is always albuminous, so that it may sometimes be difficult to decide in a given case whether the albumin found is due solely to the presence of blood or whether the hæmaturia is complicated with an albuminuria *per se*. Frequently it is possible to arrive at some conclusion by comparing the amount of albumin with the number of the red corpuscles, the presence of a large amount of the former in the presence of only a small number of the latter indicating that the albumin is not altogether due to the blood. At other times it is impossible to gain information in this manner, when the only expedient left is to determine the quantity of albumin and of iron separately, and to ascertain whether the amount of iron found is sufficient to combine with that of the albumin. As a rule, however, the presence of serum-albumin, aside from that contained in the blood of the urine, may be inferred whenever tube-casts are present, although the amount can only be estimated approximately in this manner.

Tube-casts.—In various pathological conditions, and it is claimed even in health, curious formations are seen in the urine, which represent moulds of different portions of the uriniferous tubules. To these the term *tube-casts* or *urinary cylinders* has been applied, and it may be said that there is hardly a subject of greater importance in urinary analysis, from a clinical point of view, than that of *cylindruria ;* but it must also be admitted that notwithstanding numerous investigations our knowledge of their nature and mode of formation is still

[1] Griffith, Milligan, and Forbes, Brit. Med. Jour., June 29, 1901.

defective, and the same may be said of their clinical significance. The term "tube-casts," however, is not altogether appropriate, as it is applicable to only one great division of such formations—*i. e.*, to those consisting of a uniform, transparent, gelatinous matrix, to which other elements, such as epithelial cells, red blood-corpuscles, leucocytes, and salts in a crystalline or amorphous form, may accidentally have become attached—*the tube-casts proper.*

From these the so-called "pseudocasts" must be sharply differentiated, a pseudocast being characterized essentially by the absence of a uniform matrix. Closely related apparently to the true casts are the so-called cylindroids—*i. e.*, band-like formations which resemble the former in appearance, and like these may carry various morphological elements as well as salts. It is thus necessary to distinguish between true casts, pseudocasts, and cylindroids. Of these, the true casts are by far the most important. They may be divided into hyaline and waxy casts, the two forms being readily differentiated by the fact that the former readily dissolve in acetic acid, while the waxy casts are either not affected at all by this reagent, or, if so, at least not so rapidly. The latter, moreover, are more strongly refractive, to which property their waxy appearance is due; their color is slightly yellow or yellowish gray, while the hyaline casts are colorless and usually very pale and transparent.[1]

Mode of Examination.—Unless a urine can be examined within a few hours after being voided, it is well to add a small amount of chloroform, so as to guard against bacterial decomposition. The use of conical glasses is unsatisfactory, and I find it more convenient to keep the urine in well-stoppered bottles. Preserved with chloroform it will keep almost indefinitely. Where a centrifugal machine is available the specimen can, of course, be examined at once. As soon as a sufficient amount of sediment has been obtained, a few drops are spread on a slide and examined, *uncovered, with a low power. It is essential, however, to make use of the flat mirror and to avoid a bright light. If this is borne in mind, no difficulty whatever will be found in demonstrating even the most hyaline specimens, though they may be present in very small numbers.* In many textbooks on urinary analysis the writers speak of the difficulty attending the search for hyaline casts, and the advice is frequently given to color the preparations with a drop of a dilute aqueous solution of iodo-potassic iodide, or of some other staining reagent, such as gentian-violet, picrocarmin, methylene-blue, or osmic acid. This is unnecessary if the directions just given are strictly followed. If a *bright* light is used, however, I am willing to admit that even the most experienced examiner may be unsuccessful in his search.

For the preservation of mounted specimens the following method,

[1] Rovida, see J. Moleschott, Untersuchung. z. Naturlehre d. Menschen u. d. Thiere, 1867, vol. xi., I. p. 182.

devised by Krönig, may be employed, though I personally prefer to keep the urine itself and to mount a fresh specimen when necessary. A drop of the sediment, best obtained by centrifugation, is spread on a cover-glass and allowed to dry in the air. It is then placed in a 10 per cent. solution of formalin, for ten minutes, rinsed in water, and stained for about ten minutes in a concentrated solution of Sudan III. in 70 per cent. alcohol. The excess of stain is removed by immersion for one-half to one minute in 70 per cent. alcohol, when the specimen is counterstained with Ehrlich's hæmatoxylin, rinsed in water, and mounted in glycerin. Evaporation is guarded against by ringing the specimen with asphaltum. The tube-casts are thus stained a more or less pronounced blue, the nuclei of the leucocytes dark blue, and any fatty granules or needles of fatty acids that may be present a bright red.

I have obtained very satisfactory results by pouring a small amount of a 1 per cent. aqueous solution of eosin into one of the tubes of the urinary centrifuge, filling up with urine and then centrifugating. The supernatant fluid is poured off and the sediment mixed with Farrant's solution; the specimens are finally ringed with asphaltum and keep for a long time. The hyaline casts appear a delicate rose; any adhering granules or cells are stained an intense red. Leucocytes and red cells are also well stained in this manner.

The *Farrant solution* is prepared as follows: Equal parts of distilled water, glycerin, and a saturated solution of arsenious acid (saturated during several weeks' standing) are mixed, and an amount of gum arabic added to occupy one-half of the bulk of the total mixture. This is allowed to stand for several weeks (three), stirring daily, until all the gum has dissolved. If necessary, it is then filtered, which, however, is very tedious.

Liebmann[1] recommends a mixture of 2 grammes of methylene-blue dissolved in 100 c.c. of a 10 per cent. solution of formalin. The urine is first centrifugated, the supernatant fluid is poured off, when a few drops of the reagent are poured on the sediment, and left a few minutes. The tube is filled with water, left for awhile for the salts to dissolve, then centrifugated again, when the formed elements are ready for microscopical examination.

True Casts.—1. *Hyaline Casts* (Plate XXI.).—Upon careful examination it will be seen that with rare exceptions the matrix of hyaline casts is not *altogether* homogeneous, as small granules may almost always be detected imbedded in or adhering to the matrix. As these granules occur in greater or less numbers, hyaline casts are spoken of as being finely granular (Plate XXI.), coarsely granular, finely dotted, etc. Should true morphological elements be detected, the casts are termed blood-casts, epithelial casts (Fig. 132), or pus-casts (Fig. 133). It would be better, however, to add the term

[1] P. Liebmann, Hospitalstidende (Copenhagen), July 30-Aug. 20, 1902. Abst. in Jour. Am. Med. Assoc., Sept. 20, 1902.

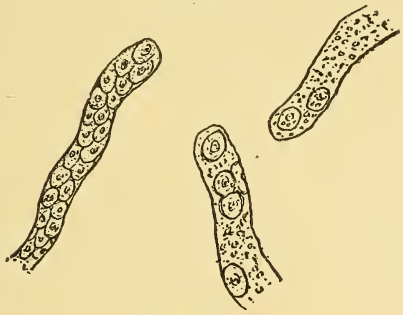
PLATE XXI.

L.S.

Hyaline, Finely Granular, and Coarsely Granular Casts.

hyaline in every instance, so as to distinguish them from pseudo-casts, which consist of these elements entirely, and lack a uniform matrix. It would thus be proper to speak of hyaline epithelial casts, hyaline blood-casts, etc., and to apply the collective term—compound hyaline casts—to these various subvarieties.

FIG. 132.



Epithelial casts.

The nature of these various forms can probably always be made out without much difficulty, and even in those cases in which the hyaline matrix is apparently concealed beneath cellular elements it will usually be possible, upon closer observation, to detect a fine boundary-line at some portion of the structure. Not infrequently

FIG. 133.

Pus-casts.

also the end of the cast will be seen to be more or less distinctly hyaline. In others, again, a hyaline zone may be observed along the sides of a central organized thread, so to speak, this being frequently seen in specimens which are very broad and long. Should doubt arise, however, a drop of acetic acid is added to a drop of the sediment on the slide; the acid dissolves the hyaline matrix, the organized constituents are set free, and the differential diagnosis between a pseudocast and a compound hyaline cast is thus readily established.

The length of hyaline casts varies greatly. It may scarcely exceed the breadth, on the one hand, while on the other, although

rarely, the cast may traverse the entire microscopical field. In breadth they vary between 0.01 and 0.05 mm. As a rule, the breadth of a cast is uniform throughout its entire length, but specimens are not infrequently observed in which one end tapers considerably and presents a spirally twisted appearance. This may be so marked that the entire cast appears transversely striated. It is generally supposed that this results from the adhesion of one end of the cast to the walls of a tubule, the lumen of which it does not fill, so that the free end becomes twisted in the downward course. A dichotomous branching of one end is also at times seen in very broad hyaline specimens.

"Fatty globules are found upon the surface of granular casts (Fig. 134), but they also form by themselves short, strongly refractive casts, which are often beset all over with needles of fatty crystals. These, however, are not composed exclusively of fat, but probably to some extent of lime and magnesium salts of the higher

FIG. 134.

a, Fatty casts. *b* and *c*, Blood-casts. *d*, Free fatty molecules. (ROBERTS.)

fatty acids and allied compounds, for they are not all soluble in ether. They have their origin doubtless in fatty degeneration of the renal epithelium" (v. Jaksch).

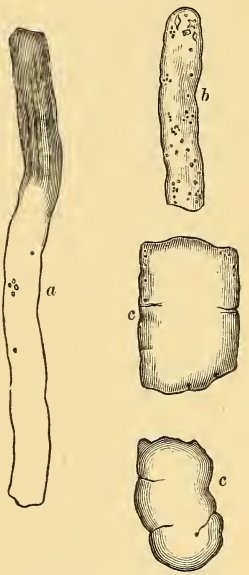
Granules of melanin, indigo, and altered blood-pigment may at times be observed in casts. Riedel regards the occurrence of dark-brown casts as pathognomonic of fractures.

2. The *waxy casts* (Fig. 135) may be divided into two groups—true waxy casts and amyloid casts; but as the latter are not necessarily indicative of the existence of amyloid degeneration of the kidneys, such a classification is at the present time at least of only theoretical interest. They are readily distinguished from the hyaline

casts by the characteristics mentioned above—*i. e.*, their higher degree of refraction, their yellow or yellowish-gray color, and the fact that they are either not attacked at all by acetic acid or only very gradually. As a rule, only small fragments are found, but these are broader and more compact than the largest hyaline casts. Waxy casts may also contain cellular elements, crystals, and amorphous mineral matter; but, as a rule, such compound casts are not so commonly observed as are those of the hyaline variety. From the latter they differ furthermore in frequently presenting a cloudy appearance, which in some cases is undoubtedly due to the presence of innumerable bacteria, and it has been suggested that these may be directly concerned in their production.

As has just been stated, some waxy casts give the amyloid reaction—*i. e.*, they assume a mahogany color when treated with a dilute solution of iodo-potassic iodide, which changes to a dirty violet upon the addition of dilute sulphuric acid. It should be remembered, however, that this reaction in casts does not necessarily indicate the existence of amyloid disease of the kidneys, as the reaction may be absent on the one hand in this condition, and present on the other where amyloid degeneration does not exist. This curious phenomenon is usually explained by assuming that such casts have remained in the uriniferous tubules for a long time, and have there undergone certain chemical changes analogous to the so-called "amyloid metamorphosis" of old precipitates of fibrin, and it is indeed possible that waxy casts are originally hyaline. Frerichs has pointed out that fibrin which has remained in the uriniferous tubules for a long time becomes denser and yellowish in appearance, which would explain the fact that these casts are only with difficulty attacked by acetic acid.[1]

FIG. 135.



Different forms of waxy casts: *a*, With a coating of urates. *b*, Waxy cast covered with crystals of calcium oxalate. *c*, Fragments of waxy casts. (V. JAKSCH.)

Before leaving this subject it should be stated that "cast-like" formations consisting entirely of amorphous urates are not infrequently encountered in urines, and according to Leube they may be obtained from any urine if it is concentrated in a vacuum at a temperature of 37° to 39° C.[2] Students frequently regard such formations as coarsely granular casts, an error which may be guarded against if the characteristics of hyaline casts set forth above are borne in mind.

[1] Rovida, loc. cit. Kobler, Wien. klin. Woch., 1890, vol. iii. pp. 531, 557, 574, 576.
[2] Leube, Zeit. f. klin. Med., 1887, vol. xiii.

Bacteria (in cases of infectious pyelonephritis), hæmatoidin, and granular detritus frequently occur grouped in a cast-like manner; their nature is readily ascertained, as in the case of the so-called urate casts just described.[1]

Pseudocasts, consisting of epithelial cells or blood-corpuscles and fibrin, are not often found in urinary sediments. The epithelial pseudocasts are probably seen only in cases of desquamative nephritis, and, unlike true casts, are hollow, the epithelium of the uriniferous tubules being thrown off *en masse*. Blood-casts (Fig. 134) consist of fibrin, within the meshes of which red corpuscles are generally found; these either present a normal appearance or occur as mere shadows, owing to the fact that their hæmoglobin has been dissolved. They are seen whenever extensive hemorrhage has taken place in the renal parenchyma, and are far more frequently observed than the epithelial pseudocasts. Hyaline casts are probably always met with in urinary sediments in which pseudocasts are found, and may be readily distinguished from the latter even when beset with numerous epithelial cells or red corpuscles (see above).

Cylindroids (Fig. 136) resemble hyaline tube-casts somewhat in general appearance, but differ from them in being much larger and band-like. Like true casts, they have a uniform breadth, and are often beset with crystals and cellular elements, such as leucocytes, red corpuscles, and epithelial cells. They are readily dissolved by acetic acid, thus differing from the *mucous cylinders* or pseudo-cylinders (Fig. 137) which may be observed in any urine containing mucus; the latter probably never contain morphological or mineral constituents, and are never of uniform breadth throughout their length. The cylindroids proper are undoubtedly of renal origin and closely related to true casts; formations are indeed not infrequently seen in which a tube-cast terminates in a cylindroid at one or both ends.[2]

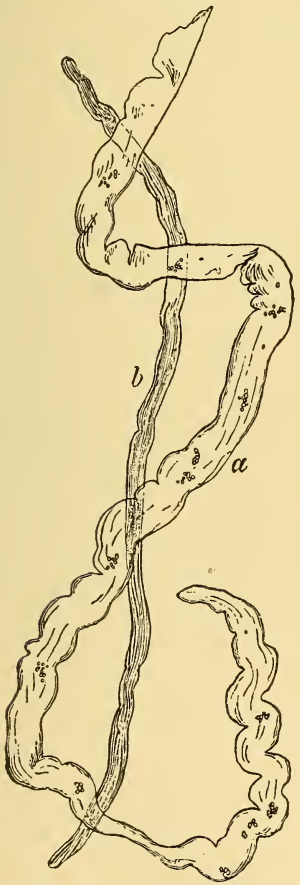
Formation of Tube-casts.—Several hypotheses have been advanced to explain the formation of tube-casts—reference is here had only to true casts, and not to pseudocasts, the origin of which is sufficiently obvious—and until recently it was quite generally accepted that they consist of coagulated albumin which has transuded into the tubules. According to this view, a cylindruria would always be indicative of the existence of albuminuria. In Neubauer and Vogel's *Urinary Analysis* (ninth edition) it is stated that "as to the significance of tube-casts, it must be remembered that these, according to our present knowledge, consist of albumin, which coagulates under the influence of the acid reaction of the urine, in the renal parenchyma, in a peculiar hyaline manner. They represent merely a

[1] Martini, Arch. f. klin. Chir., 1884, vol. xvi. p. 157. v. Jaksch, Deutsch. med. Woch., 1888, vol. xiii. Nos. 40 and 41.

[2] Bizzozero, loc. cit. Thomas, Arch. f. Heilk., 1870, vol. xi. p. 130. Pollak u. Török, Arch. f. exper. Path. u. Pharmakol., 1888, vol. xxv. p. 87.

solidified portion of the albumin held in solution by the urine; their elimination essentially indicates the existence of an albuminuria." More recently, however, it has been suggested that tube-casts are the product of a faulty metamorphosis, or of inflammatory irritation of the renal epithelium, and that a secretion from these cells or

FIG. 136.



a and *b*, Cylindroids from the urine in congested kidney. (V. JAKSCH.)

FIG. 137.

Mucous cylinders.

a disintegration of their protoplasm occurs, which results in the formation of cylindroids or true casts.[1]

Clinical Significance of Tube-casts.—Formerly the occurrence of tube-casts in urine was held to indicate the existence of nephritis.

[1] See also Rindfleisch, Lehrbuch d. path. Gewebelehre, Leipzig, 1875, p. 438. Langhans, Virchow's Archiv, 1879, vol. lxxvi. p. 85. Rovida, loc. cit. Kobler, loc. cit. Ribbert, Centralbl. f. d. med. Wiss., 1880, vol. xix. p. 305.

This view has been abandoned, however, for the same reasons which led to the rejection of the theory that albuminuria invariably indicates Bright's disease (see above).

The statement is frequently made in text-books that tube-casts may occur in the urine of perfectly healthy individuals, following severe muscular exercise, cold baths, etc.—in short, all stimuli which may cause the appearance of albumin in apparently normal individuals. It has been indicated elsewhere (see Functional Albuminuria), however, that such stimuli cannot be regarded as "physiological" in every instance, and *the presence of tube-casts in the urine similarly should be regarded as a pathological event.*[1]

It is not necessary in this connection to enumerate the various diseases in which cylindruria is observed, as they are the same as those which give rise to albuminuria; and just as a *nephrangiogenic albuminuria* is more frequently observed than a *nephritidogenic albuminuria*, so also is the presence of tube-casts in the urine more frequently due to circulatory disturbances in the kidneys than to true nephritis. In every case in which tube-casts occur in the urine it may be assumed that the accompanying albuminuria is, to a certain extent at least, of renal origin.

While the existence of cylindruria is not necessarily associated with definite pathological alterations of the renal parenchyma, this statement should be restricted to the occurrence of purely hyaline casts, and their presence in only small numbers. A few renal epithelial cells may be found at the same time, occurring either free in the urine or adhering to the casts, but never presenting an atrophic or otherwise altered appearance in the absence of definite renal lesions. The presence of compound hyaline and coarsely granular casts, as well as of waxy and amyloid casts, on the other hand, may probably always be regarded as indicating definite changes in structure, so that, so far as the diagnosis of nephritis is concerned, a microscopical examination of the urine will furnish information of more value than the simple demonstration of albumin.

Hyaline casts are those most frequently seen,—reference is here had only to the purely hyaline or, at least, but faintly granular form,—and are found in all conditions in which albuminuria occurs. When present in only small numbers, and particularly when occurring but temporarily in the urine, it may be assumed, in the absence of other symptoms pointing to renal disease, that we are dealing with a mild circulatory disturbance of the kidneys. Renal epithelial cells are absent, or present, in only small numbers. The albuminuria at the same time is trifling. If, however, hyaline casts are continuously present in large numbers, and if the amount of albumin exceeds a trace, the existence of a nephritis may usually be inferred.

[1] Nothnagel, Deutsch. Arch. f. klin. Med., 1874, vol. xii. p. 326. Burkhart, Die Harncylinder, 1884. Fischel, Prag. Vierteljahrschr., 1878, vol. cxxxix. p. 27.

In such cases granular casts and compound hyaline casts, particularly the former, will be found if the nephritis is chronic, while in the acute form the hyaline type prevails. Should blood-casts be present at the same time, the probabilities are that we are dealing with an acute nephritis or an acute exacerbation of a chronic process; in the latter case coarsely granular casts will also be present in large numbers.

Waxy casts always indicate a chronic or, at least, a subacute process. The fatty casts described by Knoll and v. Jaksch "are most commonly associated with subacute or chronic inflammations of the kidney of protracted course, with a tendency to fatty degeneration of the renal tissue. Post-mortem examination has shown that they form most frequently in cases of large white kidney. In some cases in which they were present, however, the organ was found to be more or less contracted; but when this was so, it was invariably far advanced in fatty degeneration."

It has been stated that from a careful examination of the renal epithelial cells it is often possible to determine whether an inflammatory process affecting the kidneys is at the same time complicated with degenerative changes. As a matter of fact, the cells found on the tube-casts under such conditions no longer present a normal appearance, but are shrunken and atrophied, and in cases of fatty degeneration studded with fatty granules. Epithelial casts, in the absence of distinct changes affecting the renal parenchyma, are probably never seen.

The occurrence of *pus-casts* presupposes the existence of suppurative inflammation in the kidneys, while the presence of only a small number of leucocytes on hyaline casts may be observed in the ordinary forms of nephritis, and particularly in the acute form.

The pathological significance of the so-called amyloid casts and pseudocasts has already been considered.

Cylindroids are present whenever hyaline casts are seen, and have essentially the same import. They are said to occur most frequently in the urine of children.

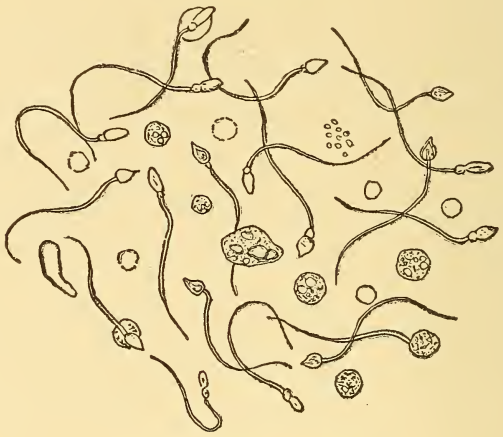
So far as the constancy with which tube-casts occur in the urine in nephritis is concerned, it is well known that in the chronic interstitial form of the disease they, as well as the albumin, are frequently absent for a long time, so that it may only be possible to make the diagnosis from the clinical history and the physical signs. It is a well-known fact, moreover, that pathological alterations of the kidneys, particularly in men past middle age, are observed again and again in the post-mortem room, where a previous examination of the urine showed no evidence of the existence of renal disease. In the acute and subacute forms of nephritis, as well as in the ordinary parenchymatous form, tube-casts are probably always found, and it would further appear that acute circulatory disturbances affecting

the renal parenchyma quite constantly lead, not only to albuminuria, but also to cylindruria.

Spermatozoa.—Spermatozoa, for a description of which the reader is referred to the chapter on the Semen, are frequently observed in the urine of healthy adults, and are quite constantly met with in the first urine passed after coitus or nocturnal emissions, when their presence is, of course, of no significance (Fig. 138). Such urines are always cloudy, but it is impossible to recognize the source of the turbidity by simple inspection.

A sediment composed of phosphates is popularly often regarded as due to semen, and no doubt every physician has seen patients, —usually sexual neurasthenics,—who were greatly alarmed at finding a white deposit in the chamber, and who imagined themselves

FIG. 138.



Human spermatozoa.

"sufferers from loss of manhood." The microscope is necessary in every case to determine the presence of spermatozoa.

In females semen may be found in the urine whenever the external genitals have been polluted during or after coitus, as well as in the exceptional cases in which connection has been effected by the urethra. From a medico-legal standpoint the discovery of spermatozoa in the urine of women may be of the greatest importance, but otherwise it is without significance.

In a few instances it is stated that trichomonads have been mistaken for spermatozoa. I am convinced, however, that such an error can only occur if the observer is totally unacquainted with the subject under consideration.

In pathological conditions spermatozoa are not infrequently found in the urine. In cases of obstinate constipation, owing to pressure

of hard scybalous masses upon the seminal vesicles, a partial evacuation of semen may occur, which may or may not be accompanied by sexual excitement. Horowitz has pointed out that a discharge of semen may be noted in cases of peri-urethral abscess with perforation into the ejaculatory ducts, giving rise to *spermato-cystitis*, the condition being due to a tight stricture of the urethra with dilatation beyond the constricted portion. I have observed a case of cystitis in which spermatozoa could almost always be detected in the urine. An operation revealed a tight stricture of the urethra and a sacculated bladder; the constant passage of semen was apparently owing to the irritating action of the ammoniacal urine. It should be noted that in this case, as well as in those in which semen is frequently passed during the act of defecation in the absence of sexual excitement, no deleterious effects referable to such loss were noted. In the urine voided during and after epileptic and, more rarely, hystero-epileptic seizures spermatozoa may be found. Such an event is undoubtedly due to muscular spasm, and is identical in origin with the emission of semen observed so frequently in the death agony, and especially during strangulation.

In certain spinal diseases semen may be found in the urine, and Fürbringer relates a case in which, following fracture and dislocation of the vertebral column, with partial destruction of the middle dorsal cord, spermatorrhœa associated with partial erection occurred thirty hours later, and continued until death, which took place after three days.

More important is the loss of semen noted in cases of true *spermatorrhœa* due to venereal excesses or masturbation, when spermatozoa may be found almost constantly, and the diagnosis indeed will often be dependent upon such an observation.

So far as the question of *sterility* in the male is concerned, reliance should not be placed upon an examination of the urine, but the semen should be obtained as soon as possible after ejaculation, and examined as indicated elsewhere.

Parasites.—Vegetable Parasites.—It has been shown by numerous investigations that bacteria are always present both in the male and female urethra, and that they may *at times* gain entrance to the bladder. The weight of evidence, however, is in favor of the view that the urine *intra vesicam* is under normal conditions free from micro-organisms, and that any bacteria which may have found their way into the bladder are rapidly killed in healthy individuals. In every urine, on the other hand, that has been exposed to the air, bacteria are always present. Whenever, then, it is desired to determine whether or not the urine of the bladder contains micro-organisms, every precaution should be taken to guard against accidental contamination. To this end, the following method should be employed: if the patient is a male, he is instructed to hold his urine

until a fairly large amount has accumulated. The glans is then thoroughly washed with soap and water, and further cleansed with cotton soaked in mercuric chloride solution (1 : 1000). The fossa navicularis is also thoroughly cleansed with the same solution. The urine is then voided under as great pressure as possible. The first portion (about 100 c.c.) is thrown away, and the second received in a sterilized vessel, when cultures should be made at once, agar or gelatin plates being inoculated with 1 or 2 c.c. of the urine. In the female the vulva is cleansed with soap and water, and the urethral aperture disinfected with bichloride solution. After then washing with sterilized water and drying with sterilized cotton the urine is evacuated through a sterilized metallic or glass catheter, and received in a sterilized vessel. Brown describes the method which is in use in Dr. Kelly's department at the Johns Hopkins Hospital as follows: the external urethral orifice being carefully cleansed with mercuric chloride solution, followed by sterile water, a sterilized glass catheter, whose external end is covered by a sterile rubber cuff, extending several centimeters beyond the end of the catheter, is introduced, the fingers of the operator being allowed to touch only the distal end of the rubber cuff. The urine is allowed to flow for a short time, when the rubber cuff is pulled off by traction on its distal end. A small amount of urine is then collected in a sterile test-tube, and the cotton plug immediately inserted. Brown states that an extended series of experiments with normal urines has shown that this method is absolutely reliable.[1]

Fig. 139.

Micrococcus ureæ.

Of the bacteria which may be found in every urine that has been exposed to the air, the *Micrococcus ureæ* is of special interest, as ammoniacal fermentation is largely due to its presence. When fermentation has commenced, it is readily recognized, occurring in almost pure culture upon the surface of the urine, mostly in the form of characteristic chains (Fig. 139). The individual coccus is colorless and quite large, so that it may be mistaken by beginners for a blood-shadow.

It is a common error to infer from the occurrence of ammoniacal decomposition very soon after micturition that this process has already begun in the bladder. It should be remembered that urine may undergo fermentation, particularly in warm weather, shortly after having been voided, and especially if the vessel employed is not perfectly clean and the urine has been exposed to the air. The diagnosis of ammoniacal fermentation in the bladder should hence only be made when the presence of ammonia can be demonstrated in the urine immediately upon being voided.

Under pathological conditions various pathogenic bacteria may be

[1] T. R. Brown, loc. cit.

found in the urine. Their presence usually indicates the existence of definite changes in the renal parenchyma, although these changes are not necessarily of an inflammatory character. Pyogenic cocci are especially prone to settle in the kidneys, and there give rise to focal inflammations; but even in the absence of such lesions they are frequently found in the urine. In all forms of infectious nephritis an abundant elimination of bacteria may generally be observed. v. Jaksch states that in erysipelas the bacteriuria and nephritis disappear, together with the cessation of the disease, and in various suppurative processes taking place in the body the specific bacteria disappear from the urine within twenty-four to forty-eight hours after evacuation of the pus.

Most interesting observations on the occurrence of bacteria in the urine of nephritic patients have been reported by Engel. Thirty-one cases were examined. In sixteen the Staphylococcus albus and aureus were found, in eight pyogenic streptococci, in four the tubercle bacillus, in five the Bacillus coli communis, and in one the typhoid bacillus, while negative results were obtained in only two instances. In the same series Engel also found a pyogenic coccus in seventeen cases. This coccus was larger than the known forms; it could be stained according to Gram's method, and did not liquefy gelatin. Intravenous injections of large numbers of the organism caused nephritis in rabbits.

In pneumonia and pneumococcus infections in general the corresponding diplococcus may be found, and in erysipelas and streptococcus infections streptococci. Very common is the presence of the Bacillus coli communis in cases of pyelonephritis; it is usually found in pure culture, but is at times associated with the Staphylococcus aureus and the Proteus vulgaris. In some instances the latter organism has also been met with in pure culture. In scarlatina streptococci have been found in a large percentage of cases; the urine was then more often albuminous than non-albuminous.

Of great interest is the frequent occurrence of the *typhoid bacillus* in the urine of typhoid fever patients. Bouchard[1] in 1881 drew attention to the elimination of the bacillus through this channel, and stated that he was able to demonstrate its presence in 50 per cent. of his typhoid fever cases. Other observers were less successful, but with improving technique and more general investigation a larger number of positive results is being obtained every year.[2] At the present time it may be said that the typhoid bacillus can be found in the urine of from 20 to 30 per cent. of all typhoid fever patients. The organism usually appears in the second or third week of the disease, and may persist for months and even years. When present

[1] Bouchard, Rev. de Méd., 1881, p. 671.

[2] For an account of the literature, see T. R. Brown, "Cystitis due to the Typhoid Bacillus," etc., Med. Record, March 10, 1900.

it usually occurs in pure culture, and often the bacilli are so numerous as to render cloudy a freshly voided specimen of urine. Symptoms of cystitis and marked renal involvement often occur, but in a considerable number of cases there are no indications of local disease. The elimination of the organism in the urine is of no prognostic significance, but is important from the standpoint of prophylaxis. Of special interest is the fact that the organism may at times be found in the urine although the patient is not the subject of typhoid fever at the time. Brown [1] thus reports the case of a woman in whom a cystitis developed on the ninth day following an abdominal operation, and in whom it was thought that the typhoid bacillus was accidentally introduced by the catheter. The patient had had typhoid fever thirty-five years previously. Young [2] gives the history of a patient in whom cystitis developed during an attack of typhoid fever, owing to infection with the typhoid bacillus. The organism could still be demonstrated in the urine after seven years. A double infection with the gonococcus subsequently occurred, and four months later typhoid bacilli and gonococci were both present in considerable numbers. Cystoscopic examination showed a chronic ulcerative cystitis. Two additional cases of chronic cystitis due to the typhoid bacillus are reported.

The bacillus may be isolated and identified according to the usual methods (see page 325).

In cases of *paratyphoid* fever the corresponding bacilli may be found in the urine.

Very important further is the fact that in tubercular disease of the urinary organs *tubercle bacilli* may be found in the urine. The search for them, however, is always tedious and frequently fruitless. In suspected cases it is best to centrifugate the urine, and to spread the sediment upon slides or cover-glasses. The preparations are then fixed by heat, and are best stained with Pappenheim's reagent (see page 357). *Grethe's method*, which was formerly used to differentiate the two, is less reliable. With this method the specimens are stained with a concentrated alcoholic solution of fuchsin, the staining fluid being brought to the boiling-point on the slide. They are then washed in water and counterstained with a concentrated alcoholic solution of methylene-blue without the application of heat. The excess of stain is washed off, when the preparations are dried with filter-paper and examined as usual. As with Pappenheim's method, the tubercle bacilli are colored red, while the other morphological elements which may be present, including the smegma bacillus, are stained blue. The usual methods of staining are not admissible, as the *smegma bacillus*, which may also be present in the urine, is likewise stained, and may readily be mistaken for the tubercle bacillus.

[1] Loc. cit.

[2] H. H. Young, "Chronic Cystitis due to the Bacillus Typhosus," Maryland Med. Jour., Nov., 1901, p. 456.

PLATE XXII.

Urethral Discharge from a Case of Gonorrhœa, showing Gonococci Enclosed in Pus Corpuscles, and Lying Free in the Discharge. Stained with Methylene Blue. (Personal Observation.)

If, in suspected cases, notwithstanding repeated examination and the preparation of numerous specimens, tubercle bacilli are not found, it is best to inject a few drops of the sediment into the anterior chamber of the eye of a rabbit, and to watch for the development of miliary tubercles in the iris.

The number of bacilli which may be found in the urine in tubercular disease of the urinary organs is extremely variable. Frequently none at all are found, notwithstanding careful search; in other cases they are present in small numbers; while in still others they are extremely numerous, and are often bunched to form particles visible to the naked eye.

Isolated tubercle bacilli have also been found in the urine in cases of acute miliary tuberculosis, in the absence of renal changes; such observations, however, are rare. Foulerton and Hillier[1] claim that the tubercle bacillus, virulent to guinea-pigs, occurs in the urine in 50 per cent. of far advanced cases of pulmonary tuberculosis, and as a rule in the absence of any renal lesion. Their observation thus accords with the findings of others that the organism in question infests the blood more commonly than was formerly supposed. As a rule, however, but few organisms were obtained.

Gonococci may be found in urinary sediments enclosed in pus cells, and can be demonstrated by preparing smears and staining with a basic dye or with the eosinate of methylene-blue solution. In the so-called gonorrhœal threads they can often be found years after the infection (Plate XXII.).

In cases of bubonic plague Kitasato's coccobacillus may be found in the urine.

In cases of cystitis a great variety of micro-organisms has been met with in the urine. Among the more important may be mentioned the staphylococcus aureus, albus, and citreus, streptococci, the bacillus coli communis, the bacillus pyocyaneus, the bacillus typhosus, the proteus vulgaris, the gonococcus, etc. In many cases of cystitis organisms are found, moreover, which are apparently non-pathogenic, and are capable of causing the formation of hydrogen sulphide from certain sulphur bodies of the urine (see Hydrothionuria).

Actinomyces kernels may be observed in the urine when the disease in question has attacked the genito-urinary tract or when the organism has found its way into the urine from other organs.

In conclusion, reference should be made to the occasional occurrence of a form of bacteriuria which is not associated with any pathological process, and has hence been termed *idiopathic bacteriuria*. Of its causation and significance nothing is known, but it is possible that in these cases a few bacteria enter the bladder either through the anterior rectal wall or are eliminated through the kidneys from the blood-current. Finding a suitable medium for their

[1] A. G. Foulerton and W. T. Hillier, Brit. Med. Jour., Sept. 21, 1901.

growth in the urine, they here multiply and may thus be constantly present. Of late, the Bacillus lactis aërogenes has been found in such a case. The diagnosis "idiopathic bacteriuria" should, of course, only be made if every possible source of contamination of the urine can be definitely excluded.[1]

Urines containing bacteria in large numbers are always cloudy, and usually present an acid reaction when voided unless cystitis exists at the same time. Attention is directed to their presence by the fact that such specimens cannot be cleared by simple filtration.

Yeast-cells in large numbers are usually only seen in urines containing sugar. Whenever a chemical examination has not been made their demonstration will be of importance, as suggesting the possible existence of glucosuria.

Moulds are usually seen in old diabetic urines after alcoholic fermentation has taken place, but they may also occur, though far less frequently, upon the surface of putrid urines that have contained no sugar.

The urinary *sarcina* which is at times met with is smaller than the sarcina of the gastric contents, but closely resembles it in appearance. It is of no clinical significance.

Whenever a urine is to be examined bacteriologically, special precaution should be taken to guard against its accidental contamination. The safest procedure, of course, is to obtain the urine by suprapubic puncture. This is, however, only exceptionally necessary, and as a general rule the method of disinfection which I have described above (see page 623) will suffice.

Animal Parasites.—The organism which Hassal saw in a urine that had been "freely exposed to the air" and was alkaline, and which he termed Bodo urinarius, was in all probability an infusorial monad and of no pathological significance. Salisbury was the first to point out that the *Trichomonas vaginalis* of Donné may at times occur in the bladder, but he gave no detailed account of his cases. Künstler, Marchand, Miura, and Dock subsequently reported cases in which flagellate protozoa were found, and modern research leaves no doubt that the organisms described by these observers are identical with the trichomonas of Donné. In Miura's case the habitat of the parasite was the urethra, and an examination of the patient's wife revealed the presence of similar organisms in the vagina. Künstler's case was one of pyelitis following cystotomy. Marchand's patient had a fistula in the perineum following suppuration in the pelvis, of unknown origin; cystitis did not exist. Dock's case was associated with hæmaturia.[2] During the past few years I have seen the same organism in seven cases, two of which occurred in the practice of Dr.

[1] Roberts, "On Bacilluria," Trans. Internat. Med. Cong., London, 1881, vol. ii. p. 157. Schottelius u. Reinhold, Centralbl. f. klin. Med., 1886, vol. viii. p. 635. Ross, Baumgarten's Jahresber., 1891, vol. vi. p. 360.

[2] Dock, Am. Jour. Med. Sci., January, 1896.

W. M. Lewis, of Baltimore. Six were women, and I have no doubt that the parasite found its way into the bladder from the vagina, where it could be demonstrated in two instances. Curiously enough a history of hæmaturia was obtained from four of the six patients. In two cases the urine contained blood at the time of the examination. In one case there was evidence of nephritis; cystitis did not exist. The number of the parasites was variable, and in five cases large.

Bälz observed numerous amœbæ in the turbid urine of a girl the subject of phthisis, which he described as being of larger size than the amœba coli.

In cases of Bilharzia disease the ova of the parasite (see page 197) are probably invariably encountered in the urine together with some blood. Sometimes the entire bulk of the urine is blood-tinged, but more often only the last few drops contain blood, and in these last drops the eggs of the parasite will also be found. In doubtful cases it is always best to examine this portion. The eggs are readily seen with a low power (see Fig. 140).

Fig. 140.

A gonorrhœal thread.

Filaria embryos may be found in the urine in cases of filarial chyluria. They should be looked for in the coagulum, a bit of which is teased out and pressed between two slides.

Billings and Miller[1] have recently reported the possible occurrence of the Anguillula aceti in the urine, in cases in which the urine is collected in bottles that had contained old vinegar. The worm closely resembles the Anguillula stercoralis. Stiles has made a similar observation.

Echinococcus hooklets and fragments of cysts may also be found, and in rare instances ascarides find their way into the urinary passages when a fistulous opening exists between the rectum and the bladder. Bothriocephalus linguloïdes (Leuckart) was found in the urine in a case occurring in Eastern Asia. Eustrongylus gigas is

[1] Billings and Miller, Trans. Assoc. Am. Phys., 1902, p. 161.

likewise found very rarely. Moscato records one case in which chyluria existed at the same time. In Clark's case, which was recently reported in the United States, the passage of the worm was accompanied by hæmaturia.

Tumor-particles.—Tumor-particles are so rarely seen in the urine that a detailed account of their occurrence may be omitted, particularly as it is seldom possible to base the diagnosis of tumor upon the presence of fragments in the urine, the clinical history and the physical signs being usually sufficient to reach a satisfactory diagnosis.

Foreign Bodies.—Of foreign bodies which may be found in the urine may be mentioned particles of fat, fibres of silk, linen, and wool, etc.; in short, material the presence of which is owing to the use of unclean vessels for reception of the urine. Fecal matter may be passed by the urethra; such an occurrence, of course, always indicates the existence of an abnormal communication between the bowel and the urinary passages. Hair derived from a dermoid cyst may similarly be found. In hysteria foreign bodies of almost any kind, such as hair, teeth, fish-bones, wood, etc., and even snakes and frogs, may be shown the physician as having been passed in the urine. I had occasion to examine "gravel" "passed" from time to time by a hysterical patient in large amounts, "every attack being accompanied by agonizing pains shooting down into the lower abdomen"; the gravel upon examination proved to be mortar, obtained from the cellar of the patient's house.

CHAPTER VIII.

TRANSUDATES AND EXUDATES.

In health the so-called serous cavities of the body contain very little fluid, and quantities sufficient for analytical purposes can normally only be obtained from the pericardial sac. In pathological conditions, on the other hand, large accumulations of fluid may be observed, not only in the serous cavities, but also in the areolar connective tissue, beneath the skin, and beneath the muscles. When due to circulatory disturbances, a hydræmic condition of the blood, or an insufficient elimination of water through the kidneys, such accumulations of fluid are spoken of as *transudates*, while the term *exudates* is applied to similar accumulations of inflammatory origin.

Clinically, it is frequently difficult to distinguish between transudates and exudates, and large ovarian, pancreatic, and hydatid cysts, as well as cystic kidneys, may at times be mistaken for ascites. In such cases a careful chemical and microscopical examination of the fluid in question may be of decided value. Very frequently, moreover, it is possible *only* in this manner to determine the nature of the disease, and *the free use of the trocar and the aspirating-needle in diagnosis cannot be too strongly advocated.*

TRANSUDATES.

General Characteristics.

Transudates are usually serous in character, when they present a light-straw color ; at times, however, owing to admixture of blood, they have a reddish tinge, and are then said to be sanguineous ; in rare instances they are chylous.

Specific Gravity.

The specific gravity varies somewhat according to the origin of the fluid, but is usually lower than that of serous exudates occurring in the same cavities—one of the most important points of difference between the two kinds of fluid. Thus, in acute pleurisy the specific gravity of the exudate is usually higher than 1.020 ; and in chronic pleurisy, if an accumulation of pus exists at the same time, higher than 1.018, reaching even 1.030. In transudates into the pleural cavity, on the other hand, referable to circulatory disturbances, for

example, as in cases of hepatic cirrhosis or cardiac insufficiency, the figures obtained are usually lower than 1.015. Transudates of peritoneal origin similarly present a specific gravity varying between 1.005 and 1.015, while that of exudates frequently reaches 1.030.

As the chemical composition, in so far as the mineral constituents and extractives are concerned, is practically the same in both classes of fluid, the difference in the specific gravity appears to be essentially due to the amount of albumin present, viz., serum-albumin and serum-globulin. It may be demonstrated, as a matter of fact, that exudates contain far more albumin than transudates, the amount varying between 4 and 6 per cent. in the former, as compared with 1 and 2.5 per cent. in the latter. The largest amounts of albumin in transudates are found in those of pleural origin, while in œdema not more than 1 per cent. is usually present.

In the table below, taken from Reuss, the relation between the percentage-amount of albumin and the corresponding specific gravity is shown. Reuss suggests the following formula for the purpose of determining from the specific gravity the amount of albumin in transudates and exudates:

$$E = \tfrac{8}{8}\,(S - 1000) - 2.8,$$

in which E indicates the percentage-amount of albumin and S the specific gravity taken by means of an accurate urinometer.

Specific gravity.	Albumin.	Specific gravity.	Albumin.
1.008	0.2	1.019	4.3
1.009	0.6	1.020	4.7
1.010	1.0	1.021	5.1
1.011	1.3	1.022	5.5
1.012	1.7	1.023	5.8
1.013	2.1	1.024	6.2
1.014	2.5	1.025	6.6
1.015	2.8	1.026	7.0
1.016	3.2	1.027	7.3
1.017	3.6	1.028	7.7
1.018	4.0		

The following table shows the percentage-amount of albumin obtained by Runeberg in ascitic fluid under various pathological conditions:

	Average	Maximum.	Minimum.
Hydræmia (Bright's disease, tuberculosis, etc., with amyloid degeneration)	0.21	0.41	0.03
Portal stasis (referable to hepatic cirrhosis or stenosis)	0.97	2.68	0.37
General venous stasis (referable to organic heart-disease)	1.67	2.30	0.84
Carcinoma of the peritoneum (complicated with carcinoma of the stomach).	3.51	5.42	2.70
Chronic peritonitis (one case complicated with heart-disease)	3.71	4.25	3.36

A great deal of stress was formerly laid upon these factors in the diagnosis of transudates from exudates, but a careful study of the

available data goes to show that after all they are uncertain guides. More reliable information is gained from the chemical study of the albumins in accordance with the suggestion of Umber more especially (see page 638).

The fact that transudates do not coagulate spontaneously in the absence of blood may further serve to distinguish them from exudates, in which a coagulum is frequently observed after standing for twenty-four hours. Not much reliance should be placed upon this point of difference, however, as exudates likewise do not always coagulate, and clotting of transudates in the presence of blood may take place within the body.

LITERATURE.—Reuss, Deutsch. Arch, f. klin. Med., vol. xxviii. p. 317. Runeberg, Ibid., 1884, vol. xxxiv. pp. 1 and 266; and Berlin. klin. Woch., 1897, No. 33. Citron, Ibid., 1897, p. 854; and Deutsch. Arch. f. klin. Med., vol. xlvi. Ranke, Mittheil. a. d. med. Klin. z. Würzburg, 1886, vol. ii. p. 189.

Chemistry of Transudates.

An idea of the chemical composition of the various forms of transudates may be formed from the following tables, taken from Hoppe-Seyler and Hammarsten, the figures corresponding to 1000 parts by weight of fluid; the specimens were taken from one individual:

	Pleura.	Peritoneum.	Œdema of the feet.
Water	957.59	967.68	982.17
Solids	42.41	32.32	17.83
Albumin	27.82	16.11	3.64
Ethereal extract	14.59	5.27	0.50
Alcoholic extract		10.94	3.71
Aqueous extract			1.10
Inorganic salts			9.00
Errors of analysis			0.12

ANALYSIS OF HYDROCELE FLUID.

Water	938.85
Solids	61.15
Fibrin (formed)	0.59
Globulins	13.52
Serum-albumin	35.94
Ethereal extract	4.02
Soluble salts	8.60
Insoluble salts	0.66
Sodium chloride	6.19
Sodium oxide	1.09

Sugar and uric acid in small amounts are also, as a rule, found in transudates, and in one case of hepatic cirrhosis Moscatelli succeeded in demonstrating the presence of allantoin. v. Jaksch states that he has frequently been able to demonstrate the presence of urobilin in both transudates and serous exudates, even though red blood-corpuscles and blood-coloring matter in solution were absent. Stich

also reports that in the ascitic fluid removed during life from a patient with hemorrhagic nephritis, urobilin was present. Peptone is never found; and Pajikull states that nucleo-albumin is not present in transudates of non-inflammatory origin. Hammarsten, together with Pajikull, could, however, demonstrate an albuminous substance in transudates, which was regarded as a mucoid and which is present in exudates in small amounts only. It is rich in reducing substance and contains more nitrogen than the true mucins.

LITERATURE.—Moscatelli, Zeit. f. physiol. Chem., 1889, vol. xiii. p. 202. v. Jaksch, Zeit. f. Heilk., 1891, vol. xi. p. 440. Eichhorst, Zeit. f. klin. Med., 1881, vol. iii, p. 537. Stich, Münch. med. Woch., Oct. 29, 1901.

Microscopical Examination of Transudates.

Upon microscopical examination only a few isolated leucocytes and endothelial cells derived from the serous surfaces and undergoing fatty degeneration are usually seen. Mast-cells and eosinophilic leucocytes have been observed in the ascitic fluid in cases of myelogenous leukæmia. Charcot-Leyden crystals were present at the same time. In cases in which the transudates have been confined for a long time plates of cholesterin are frequently found. They are especially abundant in hydrocele fluid. The technique which should be employed in the microscopical examination of transudates is described below.

EXUDATES.

Exudates may be serous, serofibrinous, hemorrhagic, seropurulent, purulent, putrid, chylous, or chyloid. Of these, the seropurulent, purulent, and putrid types are manifestly of inflammatory origin, while in the case of the serous, serofibrinous, and hemorrhagic forms it may at times be difficult to determine whether the fluid represents a transudate or whether it is an exudate. A detailed chemical and microscopical examination may then be necessary.

Serous exudates are clear, of a light straw color, and present a specific gravity which usually exceeds 1.018. If blood-corpuscles are present in sufficient numbers to impart a distinct red color to the fluid, it is *hemorrhagic;* the color may then vary from a light pink to a dark red. On standing, even the purely serous exudates generally undergo a certain degree of coagulation, which becomes more marked in the presence of blood; exceptions, however, occur. Most important is the microscopical examination of the exudates. Generally speaking, the same methods are here employed as in the case of the blood, but the interpretation of the findings is not always easy. This is largely owing to the fact that the leucocytes often show evidence of degeneration, and that the fluid may

contain endothelial cells in addition to the morphological elements of the blood, which further increases the difficulties attending a proper classification (see Pus; page 641). The principal point at issue in the study of the cellular elements of exudates is the question as to the predominance of either lymphocytes or the polynuclear elements of the blood. Widal and his school, more especially, have pointed out that whereas in exudates of non-tubercular, acute inflammatory origin the polynuclear neutrophilic leucocytes predominate, the lymphocytes prevail in the chronic tubercular forms. His observations have been confirmed by numerous investigators, and the importance of *cytodiagnosis* in pleuritic effusions more especially is now well established. From the available data we may formulate the following conclusions. In the very earliest stages of tuberculosis involving the serous membranes there is found a variable number of neutrophilic leucocytes in addition to lymphocytes and endothelial cells. Very soon, however, they diminish and in the later stages the lymphocyte is by far the predominating cell, while the neutrophilic elements are present only in very small numbers. Generally speaking, the percentage of lymphocytes in tubercular pleurisies ranges from 50 to 98 per cent., increasing as the disease continues.

In pleuritic effusions due to the pneumococcus and to streptococci during the serous stages, the neutrophilic leucocytes far outnumber the lymphocytes. In the pneumococcus cases, moreover, it is common to meet with large numbers of endothelial cells, sometimes containing polynuclear leucocytes and red cells in their interior.

In cases of traumatic and aseptic pleurisy, in association with diseases of the heart and kidneys, large endothelial cells, often presenting most grotesque appearances, occurring either singly or in groups of two, three, four, or more, are practically the only morphological elements that are met with.

French writers also describe a pleural eosinophilia, in which large numbers of eosinophilic cells—6–54 per cent.—are found in the effusion, while in the circulating blood their number is not increased. Rabaut reports four cases of this kind. In one the effusion occurred secondarily in the course of syphilis; in the second in a case of typhoid fever; the third was a case of phthisis, while in the fourth no diagnosis was made.

Mast-cells are rarely seen in pleuritic effusions, and it has been observed that their granules are then quite readily soluble in water, so that they cannot be demonstrated with aqueous solutions of the usual dyes. Wolff notes a case in which the mast-cells constituted about 10 per cent. of the total number of leucocytes in a pleuritic exudate.

Whether or not the conclusions which have been reached regarding the meaning of the prevalence of certain cell forms in pleural

effusions can be directly applied in the case of ascitic fluid remains to be seen. So far the subject has received but little attention. The same is true of the cytological study of joint-effusions. Widal reports that in three cases of acute rheumatism he found polynuclear leucocytes in the serous exudate, while they were absent in traumatic cases of arthritis.

Of the cytological findings in the cerebrospinal fluid a detailed account will be given later (see page 653).

Very important also is the study of the cellular elements which are found in serous exudates in cases of malignant disease of the serous membranes. Difficulty may here be encountered in the interpretation of the cellular findings, for on the one hand it is often difficult to distinguish the endothelial cells from leucocytes, as they take on phagocytic activity and often present the most bizarre forms. The nucleus which is normally centrally located takes up an excentric position, and enclosed within the cell we may find leucocytes and red cells. On the other hand, it is impossible by simple inspection to distinguish normal endothelial cells from *cancer cells*. In cases of doubt it is well to ascertain whether the epithelial elements give the glycogen reaction and to hunt for the presence of mitosis. Quincke has pointed out that normal endothelial cells do not contain glycogen, and that a marked iodine reaction is very suggestive of carcinoma. Wolff, however, suggests that this test is probably not specific, and cites two instances in which he obtained a positive glycogen reaction, although a tumor did not exist. More important probably is the presence of mitoses. In non-malignant exudates epithelial cells never present evidence of mitosis, while in cases of tumor (sarcoma) they may be found. Rieder regards their occurrence as pathognomonic of malignant disease. Commonly the mitosis is atypical; the division of the nucleus is not followed by a division of the cell; the chromosomes are short and show no polar or equatorial arrangement.

In cases of neoplasm Quincke has also drawn attention to the occurrence of large numbers of fat droplets in the fluid, which may attain a diameter of from 40 to 50 μ. At times, however, the fat droplets are so small and so numerous as to give a chylous appearance to the exudate. At other times a similar appearance is due to the presence of minute albuminous granules, which may be readily distinguished from fat by their insolubility in ether and the fact that they are not stained with the common fat dyes, such as Sudan, scarlet-R, and alkanin. The occurrence of numerous fatty acid crystals, arranged in groups, should also excite suspicion of a neoplasm.

Should bits of tissue be obtained, a positive diagnosis of malignant disease may, of course, be made by the usual methods. Such particles should be placed at once in absolute alcohol or formalin.

Crystalline elements are not usually seen in serous or hemorrhagic exudates; at times we meet with platelets of cholesterin.

Technique.—In every case the fluid should be examined as soon after puncture as possible; if this cannot be done at once, coagulation may be prevented by the addition of sodium citrate. The material is then placed in the ice box until a sediment has collected; or this may be obtained at once by centrifugation, new portions of fluid being repeatedly used and the sediments combined. Cover-glass preparations may then be conveniently made, or smears on slides exactly as in the case of blood, care being taken to do as little injury to the cellular elements as possible. The smears should be very thin, so that the specimens will dry rapidly and but little chance is given for the cells to contract beyond their usual size. Subsequent treatment will depend upon the special points which are to be elicited. Unfortunately the leucocytes are often much changed, so that their classification may be attended by considerable difficulties. The polynuclear elements may appear mononuclear and not infrequently the neutrophilic granules can no longer be demonstrated (see page 641). For this reason the triacid stain is not to be recommended for routine work; the eosinate is much better and will furnish as satisfactory results as can be obtained with a panoptic dye. Care should be had not to diagnose eosinophilia from the fact that cell granules are stained red, as the neutrophilic granules of degenerating cells are commonly amphophilic, viz., they stain both with acid and neutral dyes; account must be taken of the size of the granules and the general structure of the cell. To differentiate pseudolymphocytes from true lymphocytes, Pappenheim's methyl-green-pyronin may be employed, though it is not absolutely specific; still it will be found that even though the protoplasm of other cellular elements may take the red color of the pyronin, the intensity is distinctly less than in the case of the lymphocytes proper.

To study *mitosis*, hæmatoxylin and eosin may be employed, or the Romanowsky method in one of its various modifications.

The glycogen reaction is demonstrated as in the case of the blood.

Bacteriological Examination of Exudates.—In a measure the bacteriological examination of exudates has been supplanted by the cytological study, as outlined above; especially as the bacteriological examination has been notoriously unsatisfactory in the most important group of effusions, viz., in those of tubercular origin. It is now known that *all* exudates gradually become free from bacteria, even though at first they may have been caused by bacterial activity. As a result it is no longer justifiable to conclude that a process is tubercular because bacteriological examination of the exudate has given no positive result. If it is desired to cultivate organisms

that may be present, it is well to make a bouillon culture in every case so as to eliminate the bactericidal properties of the exudate as much as possible. In any event it is well to centrifugate the fluid in a sterile tube and to use the sediment for inoculations. The organisms which are most likely to be encountered are the pneumococcus, the various staphylococci, streptococci, and more rarely the colon bacillus and the typhoid bacillus.

LITERATURE.—Widal and Ravaut, "Cytodiagnostique des epanchements serofibrineux de la plèvre," Trans. XIII. Internat. Med. Cong. Paris, 1900. Barjou and Cade, "Études cytol.," etc., Arch. gén. d. méd., August, 1902. Gulland, "Cytodiagnosis," etc., Scott. Med. and Surg. Jour., June, 1902, p. 490. A. Wolff, "Transudates and Exudates," Zeit. f. klin. Med., 1902, vol. xxii. Hefte 5 u. 6. Quincke, Deutsch. Arch. f. klin. Med., 1882, vol. xxx. pp. 369 and 580. Rieder, Ibid., 1895, vol. liv. p. 544.

Chemistry of Exudates.

According to Moritz, an albumin is found in exudates that can be precipitated with acetic acid and which is absent in transudates. He regards this as serum-globulin which has undergone a change as a result of the inflammatory process. According to Matsumoto, on the other hand, the substance in question represents a mixture of fibrinoglobulin, euglobulin, and a small amount of pseudoglobulin; in the filtrate, however, there is also some fibrinoglobulin (fibrinogen) and euglobulin. He suggests that this last circumstance is probably referable to the small amount of salt in exudates and that in the first instance the pseudoglobulin is probably carried down mechanically.

Still more recently Umber has studied the body in question and arrives at the conclusion that it belongs to the mucins. To its presence the mucinous character of such fluids is due. It is precipitated by the addition of acetic acid and is insoluble in an excess of the reagent unless the acid is present in great concentration. The body has markedly acid properties and is not coagulated by heat. It differs from the known mucins in the presence of a very small amount of reducing substance, which can only be demonstrated by special methods. It contains about 14 per cent. of nitrogen and no phosphorus. In neutral and feebly acid solution the substance does not coagulate (thus differing from globulins). The same body apparently was found by Salkowski in an exudate into the hip-joint. Umber calls this substance *serosamucin*. It amounts to less than 0.5 per cent.

According to Umber and Stähelin, the serosamucin is essentially found in exudates referable to inflammatory processes or associated with new growths. In transudates, as Runeberg already pointed out, only a very slight turbidity results upon the addition of acetic acid, and not in all cases, moreover, so that a well-marked reaction viz., a marked precipitation upon the addition of acetic acid to the point of a distinctly acid reaction, may be regarded as a valuable

sign in the diagnosis between transudates and exudates. I append some of the results obtained by Umber:

Ascites.

	No. of cases.	Serosamucin.
Hepatic cirrhosis	6	0
Hepatic cirrhosis with chronic nephritis and phthisis	1	0
Nephritis	1	0
Mitral disease	3	0

Pleural Exudates.

Degeneratio cordis and nephritis	2	0
Myocarditis	1	0
Hepatic cirrhosis	1	0
Lymphosarcoma (pleura intact post mortem)	1	0
Carcinoma mammæ with pleural metastases	1	+
Tuberculosis of pleura	1	+
Pleuritis exsudativa acuta	1	+
Pleuritis and pericarditis	1	+

In addition to the serosamucin and the common albumins mentioned, some exudates may possibly also contain small amounts of a nucleo-albumin, as is suggested by the findings of Pajikull. Should ovarian cysts have ruptured into the peritoneal cavity, we may further find both pseudomucin and paramucin (which see).

Isolation of Serosamucin.—To isolate the serosamucin, the following procedure may be employed (Umber): The exudate is placed in the cold (about 0° C.) for a few hours in order to let the morphological elements settle down. The supernatant clear fluid is siphoned off, filtered, and diluted with an equal volume of water. Moderately dilute acetic acid is then added cautiously until the reaction is distinctly acid. The mucin separates out and settles at the bottom as a flocculent precipitate. It is washed with water containing a little acetic acid by decantation (two to three times); then with 80 per cent. alcohol and at intervals of twenty-four hours with stronger alcohol, and finally with ether. In this manner a preparation is ultimately obtained which can be collected on a filter, while at first filtration is entirely out of the question as the substance rapidly clogs the pores of the filter. The isolated substance is practically insoluble in water and with difficulty so in alkalies and fairly concentrated acids.

Of the common albumins we meet with traces of fibrinogen and with fairly large amounts of globulins and true albumins (see page 632). Their percentage may at times not appear so very large, but considering the large amount of fluid and the rapidity with which it may accumulate it is clear that the loss of nitrogen to the body in this form may be very considerable. Umber thus showed that in one of his cases 5000 grammes of albumin, representing about 15,000 grammes of muscle tissue were lost within a year.

Of interest further is the fact that Umber has also succeeded in demonstrating the existence of autolytic processes in exudates. He

found both albumoses and mono-amido acids, viz., leucin and tyrosin. Peptones were at no time encountered.

Coriat has recently reported a case of polyneuritic delirium, in which pleurisy with effusion developed. In the effusion he could demonstrate a peculiar albuminous substance, which he regards as identical with Bence Jones albumin; in the urine this substance could not be found.

LITERATURE.—Pajikull (Swedish ref. by Hammarsten: Jahresber. f. Thierchem., 1893). Moritz, Münch. med. Woch., No. 42, 1902. Matsumoto, Deutsch. Arch., 1902, vol. lxxv. p. 409. Stähelln, Münch. med. Woch., 1902, No. 34. F. Umber, Zeitsch. f. klin. Med., 1903, vol. xlviii. p. 364. Coriat, "The Occurrence of the Bence Jones Albumin in a Pleuritic Effusion," Am. Jour. Med. Sci., 1903, vol. cxxvi. p. 631.

Pus.

General Characteristics of Pus.—If pus, which usually presents a color varying from yellowish gray to greenish yellow, is allowed to stand for a time, a liquid gradually appears at the top, and increases in amount until it is finally possible to distinguish two distinct layers, the one above—the pus-serum, the other at the bottom—the pus-corpuscles. Upon the number of the latter the consistence as well as the specific gravity of the pus is dependent. This may vary between 1.020 and 1.040, with an average of 1.031 to 1.033. Fresh pus has always an alkaline reaction, which may become neutral or slightly acid upon standing, owing to the development of free fatty acids, glycerin-phosphoric acid, and lactic acid. The color of pus-serum may be a light straw, a greenish or a brownish yellow.

Chemistry of Pus.—The chemical composition of pus-serum and pus-corpuscles may be seen from the following tables:

ANALYSIS OF PUS-SERUM.

	I.	II.
Water	913.70	905.65
Solids	86.30	94.35
Albumins	63.23	77.21
Lecithin	1.50	0.56
Fat	0.26	0.29
Cholesterin	0.53	0.87
Alcoholic extract	1.52	0.73
Aqueous extract	11.53	6.92
Inorganic salts	7.73	7.77

ANALYSIS OF PUS-CORPUSCLES.

	I.	II.
Nuclein	342.37	673.69
Insoluble matter	205.66	
Albumins	137.62	
Lecithin, Fat	143.83	75.64, 75.00
Cholesterin	74.00	72.83
Cerebrin, Extractives	51.99, 44.33	102.84

Albumoses are usually present, and are derived from the pus-corpuscles. Leucin and tyrosin are likewise frequently met with in the pus of old abscesses; and fatty acids, urea, sugar, glycogen, biliary pigments and acids (in catarrhal jaundice), acetone, uric acid, xanthin-bases, cholesterin, etc., have occasionally been observed.[1]

Microscopical Examination of Pus.—**Leucocytes.**—If a drop of pus is examined with the microscope, it will be seen to contain innumerable leucocytes, many of which in perfectly fresh pus exhibit amœboid movements. The cells in question are usually almost altogether of the neutrophilic variety, and it may be questioned whether the lymphocytes ever occur in true pus. Even in cases of lymphatic leukæmia the predominating cell in abscesses is the polynuclear leucocyte or its degeneration-forms. Mononuclear elements with basophilic protoplasm, however, are also met with, notably in the more chronic cases, but it is likely that they are derived from the connective-tissue cells and are not of hæmatogenic origin. Eosinophiles are only seen in pus under certain definite conditions, as in gonorrhœa (see below), and mast-cells also are quite uncommon.

In pus that is not perfectly fresh it is usually not possible to demonstrate the presence of neutrophilic granules. In such cells, moreover, we commonly meet with degenerative changes affecting the nuclei, such that the polymorphous nucleus in reality becomes polynuclear, while at the same time the individual fragments are distinctly pyknotic. Such fragmentation was first noted by Ehrlich in a case of hemorrhagic smallpox and in various exudates, and has subsequently also been described by Michaelis and Wolff. The degeneration may proceed to a fragmentation of the entire cell, and it may then occur that specimens are met with in which the protoplasm still contains neutrophilic granules (Ehrlich's pseudolymphocytes, see page 83). On the other hand, a form of degeneration is seen in which the nucleus does not become pyknotic, but on the contrary swells to a large size and stains rather faintly with basic dyes. In such cells the protoplasm appears as a narrow rim and the impression is gained as though the cell were in reality a lymphocyte; if at the same time the granules have been lost, the differentiation may indeed be impossible, unless transition-forms exist between the normal polynuclear neutrophile and the type in question.[2]

Owing to resorption of water from accumulations of pus of long standing, such material finally assumes a caseous aspect, and the leucocytes will be seen to have greatly diminished in size, and to have assumed an angular, shrunken appearance; it is then hardly

[1] M. Pickardt, "Z. Kenntniss d. Chemie path. Ergüsse," Berlin. klin. Woch., 1897, p. 844.

[2] L. Michaelis and A. Wolff, "Die Lymphocyten," Deutsch. med. Woch., 1901, vol. xxvii. p. 651.

possible to demonstrate the presence of a nucleus, even after the addition of acetic acid.

It is noteworthy that in cases of hepatic abscess referable to Amœba coli it is seldom possible to demonstrate any normal leucocytes, and it will be seen that under such conditions the pus consists almost altogether of granular and fatty detritus, while in liver-abscesses due to other causes the leucocytes usually present a fairly normal appearance.

Mast-cells are only exceptionally seen in pus.

Giant Corpuscles.—So-called giant pus-corpuscles, measuring at times from 30 μ to 40 μ in diameter, have been observed in abscesses of the gum, hypopyon, and in the contents of suppurating ovarian cysts, but they do not appear to have any special significance. Upon careful examination these bodies will be seen to contain one oval nucleus, usually located eccentrically within the cell, and from one to thirty or even forty pus-corpuscles.[1]

Detritus.—Fatty and albuminous detritus in variable amount may be observed in every specimen of pus, and increases with the length of time it has been confined within the body. The same holds good for the presence of free nuclei, which were formerly regarded as young pus-corpuscles, but which have now been definitely recognized as originating during the disintegration of the corpuscles.

Red Corpuscles.—Red blood-corpuscles in variable numbers are usually seen in every specimen, their appearance depending upon the length of time they have been confined. Pus-corpuscles may at times contain a red corpuscle.

In doubtful cases it is always well to search carefully for the presence of tissue-elements, as only in this manner is it possible at times to recognize the character of the morbid process. As the data of importance have been detailed in other sections of this book (viz., Sputum and Urine), it is unnecessary to recapitulate at this place.

Pathogenic Vegetable Parasites.—Of the pathogenic organisms which are of especial interest from a clinical standpoint may be mentioned the true pus-organisms, notably the Staphylococcus pyogenes aureus and the Streptococcus pyogenes; furthermore, the tubercle bacillus, the Actinomyces hominis, the bacillus of glanders, the bacillus of anthrax, leprosy, tetanus, influenza, and Fränkel's pneumococcus, etc. The majority of these have already been described, and the reader is referred for more detailed information to special works on bacteriology. In this connection it will suffice to state that, so far as pleural exudates are concerned, an absence of micro-organisms is usually indicative of tuberculosis, while the presence of Fränkel's pneumococcus in exudates forming

[1] Böttcher, Virchow's Archiv, 1867, vol. xxxix. p. 512. Bizzozero, loc. cit.

in the course of a pneumonia appears to be a favorable omen as regards the origin of the pleuritic effusion.[1]

Protozoa, with the exception of the Amœba coli, have only rarely been found. Künstler and Pitres[2] observed numerous large spores with from ten to twenty crescentic corpuscles in pus taken from the pleural cavity of a man, which closely resembled the coccidia of mice. Litten[3] observed cercomonads in fluid withdrawn from a pleural cavity. Trichomonads have been found in empyema in connection with pulmonary gangrene.

Most important in this connection is the demonstration of the Amœba coli in the pus, and in cases of liver-abscess an examination with this view should never be neglected, as the prognosis will to a large extent depend upon the results obtained. So far as the occurrence of amœbæ in *pus* is concerned, the observation of Flexner, who demonstrated their presence in an abscess of the lower jaw, shows that they should not be looked for in the pus of abscesses of the liver or lung only.

Vermes.—Of these, the filaria and hydatids are rarely observed in this country. Bothriocephalus leguloides has been found in the pleural cavity of a Chinese patient.

Crystals.—As has been stated, crystals of cholesterin are frequently found in old pus and in exudates of long standing, but are rarely seen in recent exudates. They may be recognized by their characteristic form and their chemical reactions, as described in the chapter on the Feces (page 282). Triple phosphates, fatty acid crystals, and hæmatoidin are likewise frequently seen, the presence of the latter, of course, indicating a previous admixture of blood.

The **technique** to be employed in the examination of pus is as a rule simple. Cover-glass preparations or smears on slides are prepared as in the case of the blood and are then stained according to the points that are to be elicited. For routine work, the eosinate of methylene-blue will be found very useful. If the pus corpuscles are still fairly fresh, the neutrophilic granules are readily stained; it will be noted, however, that very commonly they exhibit a more decided red, which is referable to certain degenerative changes which cause the granules to assume an affinity for acid dyes as well. Bacteria that may be present are usually well shown. If the pus is older and the cells have lost their granules, Pappenheim's pyronin-methyl-green will be found of value in the study of the mononuclear forms (see also page 136).

[1] Ludwig Ferdinand v. Bayern, Arch. f. klin. Med., 1892, vol. l. p. 1. Fränkel, Charité Annal., 1888, vol. xiii. p. 147.

[2] Künstler u. Pitres, Compt. rend. de la Soc. de Biol., 1884, p. 523.

[3] Litten, Verhandl. d. Cong. f. inn. Med., 1886, vol. v. p. 417.

Gonorrhœal Pus.

In the very earliest stages of the disease the pus contains large numbers of eosinophilic cells besides the common polynuclear neutrophiles. But at the same time and throughout the course of the disease mononuclear non-granular elements, with basophilic protoplasm, are also seen. The larger number of the latter are of the type of the large mononuclear leucocyte and transition-form of Ehrlich, but a certain percentage is also represented by the lymphocytes, both of the small and the large variety.

The neutrophilic elements in gonorrhœal pus commonly present evidence of degeneration. In some a loss of granular material has manifestly taken place, and it can be demonstrated that in most of the cells the granules are no longer absolutely neutrophilic, but have become amphophilic—that is, from a neutral mixture they take up the neutral dye, but they can also be stained with acid dyes. With the triglycerin mixture, for example, they are stained red by the eosin.

As to the eosinophiles, Owings, who has studied this problem in my laboratory, came to the following conclusions:

1. Eosinophilic leucocytes are present in gonorrhœal pus in a large percentage of cases. They may be absent, however, even when a marked hyperleucocytosis and eosinophilia exist in the blood.

2. Their number varies *pari passu* with the number present in the blood, and the percentage in the pus is never in excess of the percentage in the blood.

3. Gonococci are rarely found in eosinophilic leucocytes.

Mast-cells may also occur in gonorrhœal pus; a remarkable case is reported by Neisser, in which the pus consisted practically exclusively of such elements.

As regards the distribution of gonococci in the different cellular elements, it is noteworthy that they are principally found in the polynuclear neutrophiles, while they are less commonly seen in the mononuclear leucocytes and transition-forms. In the small lymphocytes they are not encountered, and it is uncommon to find them in the eosinophilic cells.

Generally speaking numerous gonococci, eosinophiles, and a small number of lymphocytes are found in cases of recent gonorrhœa, while during an exacerbation of a chronic process only a few cocci and numerous mononuclear elements are encountered.

The **gonococcus** (Neisser) (Plate XXII.) occurs in the form of small oval or coffee-bean-shaped granules, grouped in twos and fours resembling a German biscuit; the individual cocci measure about 1.25 μ in length by 0.7 μ in diameter. As a rule they are found enclosed within pus-corpuscles and epithelial cells; but they may

also occur free in the pus obtained from the urethra, in the vaginal discharge, and more rarely in urinary sediments as in cases of complicating prostatitis, peri-urethritis, etc. In cover-glass specimens account should be taken only of those organisms which are enclosed within cellular elements, as these alone may be regarded as characteristic. To this end a drop of the discharge is spread in a thin layer upon a slide or a cover-glass, dried in the air, and fixed by passing three or four times through the flame of a Bunsen burner. The specimens may then be stained with any one of the basic anilin dyes. In my laboratory the eosinate of methylene-blue is almost exclusively used for this purpose (see page 123). The organisms are thus colored blue, while the granules of eosinophilic leucocytes, which may be present at the same time, appear a bright red or a brownish red. After five minutes the excess of stain is washed off, the preparations are rinsed in water, dried with filter-paper, and examined with a high power.

The gonococcus is decolorized by Gram's method and can in this manner be distinguished from similar organisms that may be present. Of the four kinds of diplococci which may be found in urethritis besides the gonococcus, only two forms are similarly decolorized, and these two are only rarely seen. We may conclude that in 95 per cent. of all cases Gram's method permits a definite conclusion as to the presence or absence of the true organism. *Gram's method* is here best employed in the modification suggested by Weinrich: The preparations are fixed by drawing through the flame of a Bunsen burner and are then stained for from one to two minutes in Fränkel's carbol-gentian-violet solution (10 parts of a saturated alcoholic solution of gentian-violet to 90 parts of a 25 per cent. solution of carbolic acid). Without washing they are placed for one to three minutes in Lugol's solution (1 gramme of iodine, 2 grammes of potassium iodide, and 300 c.c. of distilled water), and again without washing in absolute alcohol, until the alcohol ceases to extract color (about one and one-half minutes); they are now washed in water, counterstained with Bismark-brown, washed, dried, and mounted. The Bismark-brown solution is prepared as follows; 3 grammes of the dye are dissolved in 70 c.c. of hot water; 30 c.c. of 96 per cent. alcohol are added; the mixture is well stirred and filtered.

Of special interest is the observation of Unna and Plato, that the gonococcus can be stained in the living leucocyte with Ehrlich's neutral red. The method employed is simple. A small drop of the fresh pus is mixed with an öse of a dilute solution of neutral red in normal salt solution (1 c.c. of a saturated aqueous solution to 100 c.c.), and examined either as hanging drop or mounted on a slide as usual. Thus prepared, a certain number of the intracellular gonococci are stained a deep red, while others are not stained; and it may be observed on warming the slide, so as to elicit amœboid

movements, that some of the gonococci which are stained so long as they remain within the granular portion of the leucocytes, are gradually decolorized when they come to lie in the homogeneous ectosarc, and are colored again on returning to the granular endosarc. Plato states that he has examined numerous other intracellular organisms, including pseudogonococci, but that he has never observed as rapid and intense staining as with the true gonococci. He therefore suggests that with neutral red it may be possible to differentiate the true gonococcus from pseudogonococci. Extracellular gonococci, as well as numerous other bacteria, are not stained even after an exposure of several days.

The organism grows best on hydrocele agar (see below). The surface colonies are pale, grayish, translucent and finely granular, with finely notched borders. In bouillon and blood-serum mixed it forms a membrane, while the fluid remains clear.

When no discharge can be obtained from the urethra, or an examination of such discharge is negative, positive results may at times still be obtained if some of the *gonorrhœal threads* are examined which may be found floating in the urine. In these the organisms can occasionally be demonstrated after months and even years have elapsed after primary infection.

Preparation of Hydrocele Agar (Cushing).—The fluid (hydrocele or ascitic) is obtained sterile, the locality of puncture being carefully sterilized by modern surgical methods, the sterile trocar covered at its external end with sterile gauze, so as not to be infected by the operator's hand, and the fluid collected in sterile flasks, the sterile stoppers being then replaced. When collected in this way it rarely becomes contaminated and may often be kept for months before using. This fluid is mixed with ordinary nutrient agar. A number of common agar slants are placed in the autoclave for five minutes. This liquefies the agar and at the same time thoroughly sterilizes the tubes and cotton stoppers. The slants are then put in a water-bath at 55° C., so as not to coagulate the albumin when mixed with the agar. The stopper having been removed from a small flask of hydrocele fluid, the top of the flask is flamed and the albuminous fluid then poured into an agar tube (the top of which has also been flamed) in the proportion of a little more than one to two. It is well to have as much of the hydrocele fluid as the future solidity of the medium will allow. Ordinary agar will allow not quite equal parts of the two. The stopper is then returned to the agar tube, which is immediately slanted. On these slants the gonococci grow most abundantly in or near the liquid which is squeezed out of the medium and collects at the bottom of the tube. Some cultures will maintain a vigorous growth after numerous transplantations, while others again grow only two or three times, or indeed once only.

LITERATURE.—Janowski, Arch. f, exper. Pathol., 1895, vol. xxxvi. p. 15. L Michaelis and A. Wolff, "Die Lymphocyten," Deutsch. med. Woch., 1901, vol. xxvii. p. 651. A. Pappenheim, Virchow's Archiv, 1901, vol. clxix. p. 72. Neisser, Centralbl. f. d. med. Wiss., 1879, vol. xvii. p. 497. J. Plato, "Ueber Gonokokkenfärbung mit Neutralroth," etc., Berlin. klin. Woch., 1899, p. 1085. E. R. Owings, "The Infectiousness of Chronic Urethritis," Bull. Johns Hopkins Hosp., 1897, p. 210. H. H. Young, Welch Festschrift, Johns Hopkins Press., 1900, p. 677.

Putrid Exudates.

Putrid exudates are observed following perforation of a gangrenous focus or of a gastric or intestinal ulcer into one of the body-cavities. At other times they are encountered in cases of neoplasm, and at times even without apparent cause. The material obtained in such cases has a brown or brownish-green color, and emits an odor which in itself indicates the character of the exudate. Microscopically, cholesterin, hæmatoidin, and fatty acid crystals, as well as degenerating leucocytes, are found. In cases in which aspiration of a higher intercostal space reveals the presence of serous fluid, while putrid material is obtained at a lower point, the existence of a subphrenic abscess should be suspected. In such cases a pure culture of the Bacillus coli communis has been obtained. The reaction of putrid exudates is usually alkaline, but an acid reaction may be obtained in cases of perforation of a gastric ulcer; the Sarcina ventriculi and saccharomyces may then also be found.

Chylous and Chyloid Exudates.

Chylous and chyloid exudates have been repeatedly observed. They are most frequently met with in the abdominal cavity (one hundred and four times out of the total number of one hundred and fifty-five, which have thus far been reported), less commonly in the pleural cavity (forty-nine times), and only rarely in the pericardial sac (twice only). Quincke believes that the two forms can be etiologically distinguished from one another by means of a microscopical examination, as the cloudy appearance in the chyloid form is usually referable to the presence of endothelial or epithelioid cells undergoing fatty degeneration. Later observations, however, have shown that the differentiation of the two forms cannot be made upon this basis, as the same anatomical lesion, such as carcinoma, may at times give rise to the formation of a chylous exudate, at others to that of the chyloid form, and both, moreover, may coexist.

Senator claimed that the presence of more than mere traces of sugar is strongly suggestive of the chylous nature of the exudate. Possibly this observation may be of some value, but it must not be forgotten that sugar is commonly met with in all forms of transudates and exudates. Only the presence of more than 0.2 per cent. is of value.

Chylous exudates in their general appearance resemble milk, while

chyloid fluid is more suggestive of pus. The turbity in both cases is usually referable to the presence of innumerable fat-globules, which are especially abundant in the chylous form. In chyloid exudates the origin of the fat from cellular elements is often apparent at once; but, as has been said, it is impossible to draw definite etiological conclusions from that difference. Some chyloid exudates contain no fat at all, and Lion has shown that the milky appearance in such cases is owing to the presence of a curious albuminous substance, belonging to the class of nucleo-albumins. Bernert, on the other hand, claims that the substance in question belongs to the globulins, and is closely associated with certain lecithins.

LITERATURE.—Quincke, loc. cit. Boulengier, Schmidt's Jahrb., 1890, vol. ccxxvi. p. 28.

CHAPTER IX.

THE CEREBROSPINAL FLUID.

According to our present knowledge, the cerebrospinal fluid is secreted by the choroid plexuses into the lateral ventricles. Passing through the foramina of Monro, the third ventricle, and the aqueduct of Sylvius, on the one hand, it reaches the fourth ventricle and enters the cistern-like subarachnoid spaces at the base of the brain, through the foramen of Magendie and the lateral clefts of the fourth ventricle. On the other hand, a certain portion of the fluid reaches the same destination directly through the cleft in the descending horn of each lateral ventricle. The larger portion of the fluid then passes upward through the subarachnoid spaces along the convexity of the brain to the Pacchionian granulations, while the smaller portion enters the vertebral canal through the subarachnoid spaces of the spinal arachnoid membrane.

Within recent years puncture of the vertebral canal has been frequently resorted to, both for therapeutic and diagnostic purposes. The practical value of this method of diagnosis is now beyond question, and it is to be hoped that ere long physicians will resort to spinal puncture in obscure cases of cerebrospinal disease with as little hesitancy as puncture of the thoracic and abdominal cavities is now practised.[1]

The *operative method* to be employed is the following: with the patient placed upon his left side,—some observers prefer the sitting posture,—and the body bent well forward, a long aspirating-needle is introduced upon a level with the lower third of the third or fourth lumbar spinous process, and about 1 cm. to the side of the median line, the needle being directed slightly upward and inward. The depth to which it is necessary to puncture will, of course, vary with the age of the patient. In a child two years of age the vertebral canal may be reached at a depth of 2 cm., while in the adult it is necessary to insert the needle for a distance of from 4 to 8 cm. As soon as the subarachnoid space is reached cerebrospinal fluid will flow from the needle. *Aspiration* should always be avoided.

Some writers have advised that the operation be performed under

[1] H. Quincke, Verhandl. d. X. Cong. f. inn. Med., 1891. A. Hand, "A Critical Summary of the Literature on the Diagnostic and Therapeutic Value of Lumbar Puncture," Am. Jour. Med. Sci., 1900, vol. cxx. p. 463. A. Stadelmann, "Klinische Erfahrungen mit d. Lumbalpunction," Deutsch. med. Woch., 1897, p. 745.

narcosis ; and without doubt this may be necessary at times, particularly when contracture of the dorsal muscles exists. In the majority of cases, however, it is not necessary.

Amount.—So far as I have been able to ascertain, no observations have been made regarding the amount of fluid which may be obtained by puncture in normal individuals. In all probability, however, this is small. Under pathological conditions the amount may vary from a few drops to 100 c.c., and even more. In general terms it may be stated that the amount is directly proportionate to the degree of intracranial pressure. Exceptions, however, are frequent. Small amounts of cerebrospinal fluid or none at all may thus be obtained when owing to the formation of a thick exudate or the existence of a cerebral tumor communication between the basilar subarachnoid spaces of the brain and those of the spinal cord has been interrupted. Whenever, then, symptoms of intracranial pressure exist, while no fluid or minimal amounts only can be obtained by puncture, the conclusion will usually be justifiable that we are dealing with a purulent meningitis or with a tumor of the brain, and more especially of the cerebellum. It should be remembered, however, that the same result may be obtained in cases of obliteration of the aqueduct of Sylvius, or when sclerotic processes involve the foramen of Magendie, which is occasionally observed in certain forms of hydrocephalus. Adhesions of the pia mater to the arachnoid and the dura mater may, by interfering with the flow of cerebrospinal fluid, also lead to the formation of hydrocephalus, but in these cases a tumor can usually be excluded, as the changes in question always develop as sequelæ to a meningitis. A serous or tubercular meningitis, as well as acute hydrocephalus and tetanus, can, however, always be excluded when only minimal amounts of fluid are obtained by puncture. The largest amounts, on the other hand, are seen in cases of serous meningitis, tubercular meningitis, and cerebral tumors, which do not interfere with the circulation of the cerebrospinal fluid. In epilepsy Pellagrini usually obtained amounts varying between 10 and 15 c.c.[1]

Appearance.—Normal cerebrospinal fluid, as well as that obtained in cases of serous meningitis, tubercular meningitis, hydrocephalus, and tumors of the brain, is perfectly clear, and as a rule colorless unless a small blood-vessel has been punctured, when the fluid may present a slightly reddish tinge. More or less pronounced yellow shades are, however, at times observed. Important from the standpoint of diagnosis is the fact that in cases of hemorrhage into the ventricles pure blood is obtained, while such a result is, of course, a mechanical impossibility in cases of epidural hæmatoma. In subdural hæmatoma, on the other hand, blood may also find its way into the subarachnoid space, but the amount is always small, and cannot be

[1] Pellagrini, La Riforma med., 1901, Ann. 17, vol. ii. p. 638.

compared with that seen in cases of ventricular hemorrhage. Whenever, then, as in traumatic cases with severe cerebral symptoms, the surgeon is confronted with the question whether or not to trephine, puncture of the subarachnoid space may furnish much valuable information. If in such cases no blood at all is found, it may be inferred that an epidural hæmatoma or a subdural hæmatoma of slight extent only exists; an operation may then be performed. If, however, pure blood is encountered, it would be justifiable to assume the existence of extensive injury to the brain-substance proper, or, in cases in which the history is obscure, an intracerebral hemorrhage with rupture into the ventricles. In such cases the idea of an operation would, of course, be entertained only under exceptional conditions. If, further, the fluid is only tinged with blood, a subdural hæmatoma probably exists, and an operation should be advised. Accidental hemorrhage, viz., hemorrhage referable to the puncture itself, can be readily recognized, as the first few drops only are then tinged with blood, or the blood appears only after the flow has been definitely established; the amount, moreover, is insignificant.

Cloudy fluid is obtained in all cases of purulent meningitis unless the disease is limited to a very small area. This is, of course, most important from a diagnostic standpoint. Cases of abscess of the brain or sinus thrombosis occur again and again in which the question as to the advisability of operative interference is largely dependent upon the presence or absence of a complicating purulent meningitis. In certain instances a satisfactory conclusion may, of course, be reached without puncture; but in many others this is impossible, and Lichtheim's dictum, that an operation should never be undertaken in such cases unless the integrity of the meninges has been established by spinal puncture, should be borne in mind.

The degree of cloudiness naturally varies in different cases, and while in some instances the character of the fluid is seropurulent, pure, creamy pus may be found in others. Generally speaking, a cloudy fluid indicates the existence of an acute inflammatory process or an exacerbation of a chronic process.

Important, furthermore, is the fact that the fluid in non-inflammatory diseases of the brain, such as tumor or abscess, rarely undergoes coagulation, while this is the rule in all inflammatory diseases. In tubercular meningitis the coagula are very delicate, and may be well compared to spider-webs extending throughout the fluid, while in purulent meningitis the coagula are much firmer.

Specific Gravity.—The specific gravity of cerebrospinal fluid normally varies between 1.005 and 1.007, corresponding to the presence of from 10 to 15 pro mille of solids. Under pathological conditions variations from 1.003 to 1.012 may be observed, the specific gravity, generally speaking, being higher in the inflamma-

tory than in the non-inflammatory diseases of the brain. From a diagnostic standpoint, however, determination of the specific gravity is of little value, as numerous exceptions to the above rule occur.

The **reaction** is always alkaline.

Chemical Composition.—An idea of the chemical composition of the cerebrospinal fluid may be formed from the following analyses, taken from Gautier and Zdarek:

Water	987.00
Albumin	1.10
Fat	0.09
Cholesterin	0.21
Alcoholic and aqueous extract, minus salts } Sodium lactate }	2.75
Chlorides	6.14
Earthy phosphates	0.10
Sulphates	0.20

Zdarek's Analysis.

Water	989.54
Solids	10.45
Organic solids	2.09
Mineral ash	8.35
Albumins	0.76
Ethereal residue	0.35
Aqueous residue	8.22
Sulphuric acid (SO_3)	0.04
Chlorine	4.24
Carbon dioxide	0.49
Potassium oxide	0.16
Sodium oxide	4.29
Mineral ash, insoluble in water	0.16
Glucose	0.10

In addition, urea is at times found, as also a substance which reduces Fehling's solution and gives rise to a brown color when boiled with caustic potash, but which neither undergoes fermentation nor forms an osazon when treated with phenylhydrazin. The substance in question is generally regarded as pyrocatechin. Its amount varies between 0.002 and 0.116 per cent. According to C. Bernard, glucose may also be present, but it is questionable whether this is the case under normal conditions (see below). Nawratzki discovered a reducing substance in his cases, which was demonstrated to be glucose; his subjects, however, were unfortunately not normal, but general paretics with fever. Pyrocatechin was absent. Zdarek[1] reports a recent case of anterior meningocele in an otherwise normal individual in which the fluid reduced Fehling's solution and gave a glucosazon with phenylhydrazin. The substance in question was dextrorotatory, the amount corresponding to 0.1 per cent. of glucose.

So far as the albuminous bodies are concerned which may be found

[1] E. Zdarek, Zeit. f. phys. Chem., 1902, vol. xxxv. p. 202.

in the cerebrospinal fluid, serum-albumin is said to be present only under exceptional conditions, while normally a mixture of globulin and albumoses is found. The question whether or not mucin may also be present is still undecided.[1]

Under pathological conditions the amount of albumin may vary considerably, and is of diagnostic importance. According to the majority of observers, the figure given in the above analysis is too high, and it is doubtful whether 1 pro mille may be regarded as normal. The lowest values have been obtained in cases of chronic hydrocephalus (traces only), meningitis serosa (0.5 to 0.75 pro mille), and tumors of the brain (traces to 0.8 pro mille); while the largest amounts have been found in chronic hydrocephalus the result of hyperæmia (1 to 7 pro mille), and in tubercular meningitis (1 to 3 pro mille). Nawratzki in recent examinations found amounts varying between 0.047 and 0.170 per cent., but the subjects of his investigation had fever at the time.

Lichtheim claims to have found glucose—by means of the phenylhydrazin test—in all cases of tumor which he examined. In cases of tubercular meningitis, on the other hand, a positive result was only exceptionally obtained. Quincke also reports that he was able to demonstrate the presence of sugar whenever the liquid obtained was sufficient in amount for the necessary tests. Unfortunately, however, he does not detail his cases. Concetti found no sugar in hydrocephalic fluid.

The experience of other observers does not agree with that of Lichtheim and Quincke; and Fürbringer,[2] who has thus far reported the largest number of spinal punctures, found sugar in only two cases of diabetes associated with tuberculosis.

According to Gumprecht, the normal cerebrospinal fluid also contains traces of cholin.

Microscopical Examination.—According to Widal, Sicard, Ravaut, and others, it is possible to make a diagnosis of the character of the morbid process in cases of meningitis from the morphology of the cells contained in the cerebrospinal fluid. Generally speaking, lymphocytes prevail if the inflammatory process is tubercular in origin, while the polymorphonuclear variety predominates in the non-tubercular cases. Exceptions to this rule, however, occur. Marcou-Mutzner[3] thus reports a case in which the prevailing cell was a polymorphonuclear leucocyte, notwithstanding the fact that, as autopsy showed, there existed a typical pulmonary and meningeal tuberculosis. He also cites a case, reported by Rendu, in which lymphocytes predominated, and in which autopsy showed fracture of the base of the skull.

[1] Stadelmann, Mitth. a. d. Grenzgebiete d. Med. u. Chir., vol. ii. Comba. Clin. med., 1899 (cited in Arch. d. Méd. d. Enfants, 1900). Lenhartz, Verhandl. d. XIV. Cong. f. inn. Med., 1900.

[2] Fürbringer, Verhandl. d. XV. Cong. f. inn. Med., 1901.

[3] Marcou-Mutzner, Arch. gén. d. Méd., Sept., 1901, p. 345.

Bendix reports 8 cases of meningitis from Minkowsky's clinic in which an examination was made in this direction. Five of these were cases of meningeal tuberculosis, and in all the cellular elements were mostly lymphocytes, while the polynuclear cells were present in only small numbers. The 3 remaining cases were due to the meningococcus intracellularis. In 2 of these the prevailing cell was the polynuclear neutrophile, but in 1 the lymphocytes were in excess. Bendix suggests that possibly the duration of the disease rather than its bacteriological character may be the deciding factor in determining the morphological findings; that in chronic cases the lymphocytes prevail, which would account for the exception, as in this case the disease had extended over several months.

It thus appears that the cytological examination of the cerebrospinal fluid does not furnish information which is practically conclusive as in the case of pleuritic exudates (which see). Nevertheless the exceptional cases are comparatively few, and in all doubtful cases a careful study of the cellular elements is demanded.

The technique to be employed in the cytological study of the cerebrospinal fluid is the same as in the case of pleural exudates (see page 637).

Very important also from a diagnostic point is the fact that pathogenic micro-organisms may be found. Lichtheim, Fürbringer, Freyhan, Dennig, and Fränkel were thus able to demonstrate the presence of *tubercle bacilli* in a fairly large number of cases of tubercular meningitis. Other observers, it is true, have been less fortunate, but the fact that Fürbringer found tubercle bacilli in thirty cases out of thirty-seven is certainly significant. Schwarz states that he obtained positive results in sixteen out of twenty-two cases, and Slawyk and Manicatide found bacilli in all of nineteen cases (sixteen times by direct microscopical examination, and three times by the animal experiment). In order to examine for tubercle bacilli, the fluid should be placed on ice for from six to twenty-four hours, until a slight coagulum has formed, when the fine, spider-web-like threads of fibrin are transferred to a cover-slip, spread in as thin a layer as possible, and stained as described in the chapter on the Sputum. If a centrifugal machine is available, the examination may, of course, be made at once; the chances of finding the bacilli are then also much greater. In every case a large number of specimens should be prepared before the search is abandoned. Only a positive result, however, is of value, and in doubtful cases recourse should be had to the animal experiment.[2]

In the diagnosis of epidemic cerebrospinal meningitis lumbar puncture is of signal value, as the *Diplococcus meningitidis intracellularis* of Weichselbaum-Jäger can be demonstrated in a large per-

[1] Fürbringer, loc. cit. Wentworth, Arch. of Pediat., Nov., 1899.

centage of cases. Councilman thus states that during a recent epidemic of the disease in Boston lumbar puncture was performed in fifty-five cases, and that in the fluid obtained the diplococci were found on microscopical examination or in culture in thirty-eight cases. The average time from the onset of the disease before spinal puncture was made was seven days in the positive cases, and seventeen days in the negative cases. The longest time after the onset in which a positive result was obtained was twenty-nine days. Similar results have also been reached by other observers.

The organism in question is a diplococcus, each half being of about the same size as the ordinary pathogenic micrococci. It is readily stained with the usual dyes, and decolorized by Gram's method. Short chains of from four to six and tetrads may at times be seen. It grows best upon Löffler's blood-serum mixture, forming round, whitish, shining, viscid-looking colonies, with smooth, sharply defined outlines, which may attain a diameter of from 1 to 1½ mm. in twenty-four hours. Their cultivation upon plain agar, glycerin-agar, and in bouillon is less reliable.

In order to obtain the best results, it is necessary to use large amounts of the exudate, and to make a number of cultures, as many of the organisms are usually dead, or at least will not grow. In ordinary cover-slip preparations they are often numerous, and are found enclosed in the polynuclear leucocytes. Their number then varies considerably. On the one hand, only one or two may be present in a cell, while in others they may be so closely packed as to obscure the nucleus.

Mixed infections are not uncommon in epidemic cerebrospinal meningitis. Councilman thus found the pneumococcus in seven cases, and Friedländer's bacillus in one. Terminal infections with staphylococci and streptococci also occur.

In other forms of purulent meningitis a large variety of organisms has been found. Wolf gives the following figures, resulting from an analysis of 174 cases, in which epidemic cerebrospinal meningitis is, however, included: in 44.23 per cent. the pneumococcus was found; in 34.48 per cent. the Diplococcus meningitidis intracellularis; in 3.45 per cent. staphylococci; in 8.03 per cent. streptococci, in 1.13 per cent. the bacillus of Friedländer; in 2.87 per cent. the Bacillus typhosus; in 1.72 per.cent. the bacillus of Neumann-Schäffer, and in 2.87 per cent. the Bacillus coli communis, the Bacillus pyogenes fœtidus, the Bacillus aërogenes meningitidis, and the Bacillus mallei, while no bacteria were found in 1.15 per cent. of the cases. In two cases Pfeiffer's influenza bacillus has also been encountered in the cerebrospinal fluid during life.

In the African sleeping sickness trypanosomes are commonly found in the cerebrospinal fluid, obtained by lumbar puncture. Castellani obtained the organism in twenty cases of thirty-four, and Bruce found it in all of thirty-eight cases (see page 191).

Toxicity.—While normal cerebrospinal fluid possesses distinct toxic properties, it has been found that in disease the toxicity may be markedly increased. Bellisari has thus shown that the fluid of individuals suffering from general paresis is more toxic than that of normal individuals, and that this toxicity is at its maximum after an epileptic seizure. Pellagrini further could demonstrate that the cerebrospinal fluid of epileptics is markedly toxic, and that that obtained immediately after a convulsion has a toxic and convulsive power much greater than that obtained at periods far removed from paroxysms.

LITERATURE.—W. T. Councilman, "Cerebrospinal Meningitis," Johns Hopkins Hospital Bull., 1898, p. 27; and Phila. Med. Jour., 1898, p. 937. W. T. Councilman, F. B. Mallory, and J. H. Wright, "Epidemic Cerebrospinal Meningitis," Am. Jour. Med. Sci., 1898, p. 252. W. Osler, "The Cavendish Lecture on the Ætiology and Diagnosis of Cerebrospinal Fever," Phila. Med. Jour., 1899, p. 26. E. Stadelmann, "Meningitis Cerebrospinalis," Zeit. f. kliu. Med., vol. xxxviii. p. 46. R. Neurath, Centralbl. f. d. Grenzgebiete d. Med. u. Chir., 1897, vol. i. J. Langer, Jahrb. f. Kinderheilk., 1901, vol. iii. p. 91. Pellagrini, loc. cit., p. 559.

CHAPTER X.

THE EXAMINATION OF CYSTIC CONTENTS.

CYSTS OF THE OVARIES AND THEIR APPENDAGES.

THE material obtained from cysts of the ovaries or their appendages varies greatly in character. On the one hand, it may be fluid, clear, of low specific gravity, and contain little albumin; while, on the other, it may be dense, viscid, and of colloid appearance. The specific gravity varies between 1.018 and 1.024, owing to the presence of a large amount of albumin.

In addition to smaller amounts of serum-albumin and serum-globulin the fluid of ovarian cysts contains a considerable quantity of another albuminous substance, which is termed *metalbumin* (Scherer) or *pseudomucin* (Hammarsten). Like Hammarsten's mucoid of transudates, it cannot be directly precipitated with acetic acid, but must be isolated as follows: The fluid in question is freed from coagulable albumins by boiling after acidifying with acetic acid; the filtrate is precipitated with alcohol, the precipitate dissolved in water, dialyzed, and then treated with acetic acid, when the pseudomucin separates out. The substance contains about 30 per cent. of glucosamin.

Paramucin is another albuminous substance which is found in colloid cysts and belongs to the mucinoid bodies. Like the true mucins and the body which occurs in exudates the paramucin is also precipitated by dilute acetic acid. According to Mitjukoff, it contains at least 12.5 per cent. of a reducing substance.[1]

Test for Pseudomucin.—The fluid is mixed with three times its volume of alcohol and set aside for twenty-four hours, when it is filtered and the precipitate suspended in water. This is again filtered and the filtrate tested in the following manner: 1. A few cubic centimeters are boiled, when in the presence of metalbumin the liquid will become cloudy, without the formation of a precipitate. 2. With acetic acid no precipitate is obtained. 3. Upon the application of the acetic acid and potassium ferrocyanide test the liquid becomes thick and assumes a yellowish color. 4. When boiled with

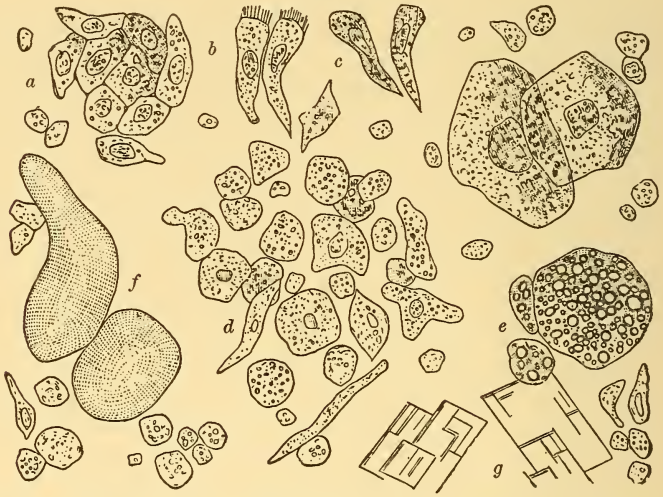
[1] Literature dealing with pseudomucin and paramucin: *Pseudomucin:* Hammarsten, Zeit. f. phys. Chem., 1882, vol. vi. p. 194. Pfannenstiel, Arch. f. Gynäk., 1890. Zängerle, Münch. med. Woch., 1900. *Paramucin:* Mitjukoff, Arch. f. Gynäk., 1895. Panzer, Zeit. f. phys. Chem., 1899, vol. xxviii. Leathes, Arch. f. exper. Path. u. Pharmak., 1899, vol. xliii.

Millon's reagent a few cubic centimeters of the filtrate will yield a bluish-red color, while the addition of concentrated sulphuric acid, without boiling, gives rise to a violet color.

The color of cystic fluids may vary from a light straw to a reddish brown, or even a chocolate; the latter color may be observed when hemorrhage has taken place into the cyst.

Of morphological elements, ovarian cysts contain red blood-corpuscles, leucocytes, and at times fatty granules in large numbers, crystals of cholesterin, hæmatoidin, and fatty acids. Most important, however, from a diagnostic standpoint is the presence of cylindrical or prismatic ciliated epithelial cells, derived from the internal lining of the cyst, in the presence of which the diagnosis may be definitely made (Fig. 141). At times such cells cannot be demonstrated, as they may have undergone fatty degeneration; moreover, if the epithelium lining the cyst is squamous in character, it may be difficult, if not impossible, to arrive at a satisfactory con-

FIG. 141.



Contents of an ovarian cyst. (Eye-piece III., obj. 8 A, Reichert.) (v. JAKSCH.)
a, Squamous epithelial cells; *b*, Ciliated epithelial cells; *c*, Columnar epithelial cells; *d*, Various forms of epithelial cells; *e*, Fatty squamous epithelial cells; *f*, Colloid bodies; *g*, Cholesterin-crystals.

clusion from an examination of the morphological elements alone. *Colloid concretions*, which may vary in size from several micromillimeters to 0.1 mm., are occasionally observed, and more particularly in colloid cysts. They may be recognized by their irregular form, homogeneous appearance, slightly yellow color, and delicate outlines.

In dermoid cysts, epidermal cells and occasionally hairs are observed.

The differential diagnosis of ovarian, parovarian, and fibrocystic

(uterine) cysts cannot always be made from the character of the fluid withdrawn by puncture, but at times it is possible. The most important points of difference are here given: 1. The fluid in ovarian cystomata is usually more or less viscid, and often contains non-nucleated granular corpuscles of about the size of leucocytes, the granules of which do not dissolve in acetic acid nor disappear when treated with ether. In all probability they are free nuclei; in the United States they are often called Drysdale's corpuscles. 2. In parovarian cysts the fluid is thin, watery, of low specific gravity (under 1.010), and contains very few morphological elements. Cylindrical epithelium is very rarely found during life in the fluid withdrawn by aspiration from either ovarian or parovarian cysts. 3. The fluid from fibrocystic tumors of the uterus is thin, watery, and coagulates spontaneously, while that from ovarian and parovarian cysts never coagulates spontaneously unless blood is present. Fibrocystic tumors of the uterus have no epithelial lining.

Of special interest are those cases of ovarian cysts in which in the course of typhoid fever infection of the cystic contents occurs with the corresponding organism.[1]

HYDATID CYSTS.

Hydatid cysts are rarely seen in the United States (see page 352). The fluid in question is clear, alkaline, of a specific gravity varying between 1.006 and 1.010, and contains no albumin. *Succinic acid* is usually present, and may be demonstrated by acidifying a small amount of the fluid with hydrochloric acid and evaporating to dryness. The residue is extracted with ether and the ether evaporated; the aqueous solution of the second residue, in the presence of succinic acid, will yield a rust-colored gelatinous precipitate when treated with a few drops of a solution of ferric chloride. *Sodium chloride* is always present in notable amounts, and may be recognized by evaporating a drop of the liquid upon a slide, when the characteristic crystals of salt will be found.[2] Most important, of course, is the microscopical examination, which may reveal the presence of hooklets and shreds of membrane, and at times of scolices (see Sputum).

HYDRONEPHROSIS.

The diagnosis of hydronephrosis can usually be made without difficulty if a sufficient amount of fluid can be obtained; the presence of urea and uric acid in *notable quantities*, as well as of renal epithelial cells, which latter especially should be sought for, is quite characteristic. *Small* amounts of uric acid, however, may also be present in ovarian cysts.

[1] M. J. Lewis and R. G. Le Conte, Am. Jour. Med. Sci., 1902, vol. cxxiv. p. 590.
[2] J. Munk, Virchow's Archiv, 1875, vol. lxiii. p. 255.

PANCREATIC CYSTS.

These cysts may be recognized by the fact that the fluid possesses the power of digesting albumin in alkaline solution. A small amount of the liquid is added to milk, when after precipitation of the caseïn the biuret test is applied; a positive reaction indicates the presence of *trypsin*. Unfortunately, however, the test does not always yield positive results, even if the fluid in question is derived from a pancreatic cyst, as the trypsin is apparently destroyed in the course of time. The larger the cyst, the less likely will it be possible to obtain the reaction. A positive result is hence only of value, while a negative result does not exclude the existence of the disease.[1]

[1] Karewski, Deutsch. med. Woch., 1890, vol. xvi. pp. 1035 and 1069. Hofmeister Prag. med. Woch., 1891, vol. xvi. pp. 365 and 377 (see Gussenbauer). v. Jaksch, Zeit. f. Heilk., 1888, vol. ix. p. 126 (see Wölfler).

CHAPTER XI.

THE SEMEN.

THE ejaculated semen is a mixture of the secretions furnished by the testicles, the prostate gland, the seminal vesicles, and the glands of Cowper.

GENERAL CHARACTERISTICS.

Semen is white or slightly yellowish in color, semifluid, sticky, and of an opaque, non-homogeneous, milky appearance, which is due to the presence of white, opaque islets floating in the otherwise clear fluid; these consist almost entirely of the specific morphological elements of the semen, the spermatozoa. Its odor, which strongly resembles that of fresh glue, is characteristic, and is owing to the presence of *spermin*. It is generally attributed to an admixture of prostatic fluid, as the semen obtained from the vasa deferentia is odorless. According to Robin, however, this odor is produced only at the moment of ejaculation, and cannot be ascribed to any single one of the secretions present. The reaction of human semen is slightly alkaline, and its specific gravity greater than that of water, in which it sinks to the bottom.

CHEMISTRY OF THE SEMEN

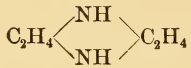
Accurate analyses of human semen or of mammalian semen do not exist, and only the old analyses of Vauquelin and Kölliker can be given:

	Man.	Horse.	Ox.
Water	90	81.90	82.10
Albuminous material	6	...	15.30
Extractives		16.45	...
Ethereal extract		...	2.20
Mineral material	4	1.61	2.60

The mineral matter consists largely of calcium phosphate.

If semen is kept, or if it is slowly evaporated, crystals of phosphate of spermin separate out, which are commonly known as Böttcher's crystals, and which were long regarded as identical with the so-called Charcot-Leyden crystals that are found in the sputum of bronchial asthma, in the blood of leukæmia, in the stools in cases of helminthiasis, etc.

Spermin is a basic substance, and, according to Ladenburg and Abel, is closely related to, if not identical with, diethylene diamin (piperazin):



The phosphate crystallizes in the form of monoclinic four-sided spindles or prisms, which appear as flattened needles of variable size. Some are scarcely visible even with a fairly high power of the microscope, while others attain the length of 40 μ to 60 μ. The substance is soluble in formol, thus differing from Charcot-Leyden crystals. In water it dissolves with difficulty; it is slowly soluble in acids and alkalies, even in ammonia, while it is insoluble in alcohol, ether, chloroform, and dilute saline solution. Florence's reagent (see below) colors the crystals a bluish black. According to Cohn, the Böttcher crystals are formed exclusively in the prostate gland, the gland itself furnishing the basic component, while the necessary phosphoric acid is derived from other portions of the reproductive apparatus.[1]

MICROSCOPICAL EXAMINATION OF THE SEMEN.

Upon microscopical examination normal semen is seen to contain innumerable actively moving thread-like bodies, measuring from 50 μ to 60 μ in length—the *spermatozoa*. These consist, of an egg-

FIG. 142.

Human semen.

a, Spermatozoa; *b*, Cylindrical epithelium; *c*, Bodies enclosing lecithin-granules; *d*, Squamous epithelium from the urethra; *d'*, Testicle-cells; *e*, Amyloid corpuscles; *f*, Spermatic crystals; *g*, Hyaline globules. (V. JAKSCH.)

shaped head, when seen from above, which is from 3 μ to 5 μ in length, the broader end being directed anteriorly; a middle portion, 4 μ to 6 μ in length, with which the head is united by its smaller

[1] Th. Cohn, "Zur Kenntniss d. Spermas," Centralbl. f. allg. Path. u. path. Anat., vol. x. pp. 940–949.

end; and a posterior piece or tail, into which the middle piece gradually fades (Fig. 142).

In addition to the spermatozoa a few hyaline bodies are seen which are derived from the seminal vesicles; further, numerous small pale granules of an albuminous nature, some testicular and urethral epithelial cells, lecithin-corpuscles, and so-called prostatic or *amyloid corpuscles*, which at first sight resemble starch-granules in appearance, owing to their concentric striations. A few leucocytes and occasionally a few red corpuscles may also be found.

PATHOLOGY OF THE SEMEN.

The study of the semen has received little attention from clinicians, and gynecologists frequently hold the wife responsible for sterility when an examination of the husband's semen would—according to Kehrer,[1] in 40 per cent.—reveal an absence of spermatozoa, constituting the condition usually spoken of as *azoöspermatism*. This may be temporarily observed following venereal excesses, when the fluid finally ejaculated is almost entirely of prostatic origin; their absence then possesses no significance, but persistent azoöspermatism must of necessity be associated with sterility.[2]

Cases have been recorded in which, notwithstanding the presence of spermatozoa and apparently normal sexual conditions in both husband and wife, sterility existed nevertheless, but in which it was observed that the spermatozoa lost their motile power almost immediately after ejaculation. Under normal conditions, following intercourse actively moving spermatozoa may be found in the vagina after hours, days, and even weeks.

Whenever it is deemed advisable to make an examination of the semen, this should be done immediately following ejaculation, or as soon as possible thereafter. Note should then be taken, not only of the presence, but also of the degree of motility of the spermatozoa; a drop of the semen is mixed with a drop of normal (0.6 per cent.) saline solution, and examined at once with the microscope.

Bloody semen, constituting the condition spoken of as *hæmospermia*, has been observed on several occasions. It may follow excessive sexual indulgence, but may also occur in connection with gonorrhœal epididymitis. The blood is readily recognized upon microscopical examination.[3]

THE RECOGNITION OF SEMEN IN STAINS.

In medico-legal cases the physician may be called upon to decide whether or not certain stains on body-linen are caused by spermatic

[1] Kehrer, Beiträge z. klin. u. exper. Gynaek., 1879, vol. ii., Giessen.
[2] Fürbringer, Zeit. f. klin. Med., 1881, vol. iii. p. 310.
[3] Feleki, Centralbl. f. Krankh. d. Harn- u. Sexualorgane, 1901, vol. xii. p. 503.

fluid, whether or not a rape has been committed, etc. In such cases it is frequently only necessary to examine a drop of the vaginal fluid in order to arrive at a positive result at once. At other times, however, recourse must be had to the following method: a fragment of the linen or scrapings from the vulva or vagina are placed in a watch-crystal and allowed to soak for at least one hour in from 27 to 30 per cent. alcohol, when a bit of the material is teased in a solution of eosin in glycerin (1 : 200), and examined. The heads of the spermatozoa are thus stained a deep red, while the tails, which are often broken, exhibit a pale-rose tint, and can readily be distinguished from vegetable fibres, which do not take the stain at all. A positive statement can thus be made in every case, even after months and years, as spermatozoa not only resist the action of reagents, but also the process of putrefaction; this is probably owing to the large proportion of mineral matter which enters into their composition, and which insures preservation of their form. Instances have been recorded in which it was possible to demonstrate spermatozoa in stains after eighteen years.

The semen test of Florence[1] has attracted much attention, and may be recommended in doubtful cases; only a negative result, however, is of value (see below). It is based upon the observation that very characteristic crystals of *iodospermin* are formed when spermatic fluid is treated with a solution of iodo-potassic iodide containing 1.65 grammes of pure iodine and 2.54 grammes of potassium iodide, dissolved in 26 c.c. of water. When a drop of this solution is added to a drop of spermatic fluid or an aqueous extract of a seminal stain, dark-brown crystals of iodospermin separate out at once, and may be readily recognized under the microscope. They occur in the form of long rhombic platelets or fine needles, often grouped in rosettes, but also occurring singly or as twin crystals. The examination with the microscope should be made at once after addition of the reagent, as the crystals disappear on standing.

As the reaction may also be obtained in cases of azoöspermatism, and with pure prostatic secretion, while a negative result is obtained with the fluid from spermatoceles, it is manifest that the test is not applicable for the determination of the presence or absence of spermatozoa *per se*. Posner[2] states that he obtained similar crystals when the test was applied to a glycerin extract of ovaries.

More recently Richter[3] has shown that Florence's reaction is also obtained with a decomposition-product of lecithin, viz., cholin,

[1] Florence, "Du sperme et des taches de sperme en médecine legale," Arch. d'Anthrop. crimin., vols. x. and xi.

[2] C. Posner, "Die Florence'sche Reaktion," Berlin. klin. Woch., 1897, p. 602.

[3] M. Richter, "D. mikrochemische Nachweis v. Sperma," Wien. klin. Woch., 1897, p. 569.

which would explain the observation that better results are commonly obtained with dried semen than with fresh material. But it follows also that the reaction cannot be a specific semen reaction, and Richter accordingly concludes that a negative result only is of value, and indicates that the material under examination is not semen. He states that he obtained positive results with vaginal and uterine mucus, with decomposing brain-substance, and other organs as well. In confirmation of Richter's results, Bocarius [1] has demonstrated that the so-called iodospermin is in reality an iodized product of cholin and not of spermin.

[1] N. Bocarius, Zeit. f. phys. Chem., 1902, vol. xxxiv. p. 339.

CHAPTER XII.

VAGINAL DISCHARGES.

GENERAL CHARACTERISTICS.

The secretion which is normally furnished by the vaginal glands is small in amount, and just sufficient to keep the mucous membrane moist. It is a clear or somewhat milky-looking, semiliquid material, in which numerous epithelial laminæ, which have been thrown off during the normal process of desquamation, may be found. It has been stated that the reaction of the vaginal secretion in virgins is *invariably* acid, while an alkaline reaction is the rule in the *déflorées*. During pregnancy, however, the secretion is probably always acid. In five hundred cases which Krönig examined in this direction an alkaline reaction was never observed. According to Zweifel, the vaginal secretion contains traces of trimethylamin.[1]

Microscopically, numerous epithelial cells, mucous corpuscles, a few large mononuclear leucocytes cellular detritus, and bacteria are found (Fig. 143). Döderlein[2] has described a non-pathogenic

Fig. 143.

Vaginal secretion.

a, Mucous corpuscles; *b*, Vaginal epithelium; *c*, Epithelium from vulva.

bacillus or a group of bacilli which are characterized by the fact that they give rise to marked acid fermentation of sugar, and he regards these organisms as the only ones which are constantly present in the normal vagina. Krönig and Menge, however, state

[1] Zweifel, Arch. f. Gynaek., 1881, vol. xviii. p. 359.
[2] Döderlein, Ibid., 1887, vol. xxxi. p. 412.

that they are often absent. These observers have found, on the other hand, that under normal conditions there are various bacilli and cocci present which belong to the class of obligatory anaërobes, and are likewise non-pathogenic. Unfortunately they have not described these organisms in detail. Near the outlet they found bacteria which may be cultivated upon alkaline aërobic culture-media, but which are usually absent in the upper portion of the vagina.

It is important to note that various diplococci may also be found under normal conditions, and care should be taken not to confound these with gonococci. Like the gonococci, they are decolorized by Gram's method. If the characteristics of the former be borne in mind, however, mistakes may probably always be avoided; in married women and in children it is best to make the diagnosis of gonorrhœa only when the gonococcus has been isolated by cultivation.

The question whether or not pathogenic bacteria *may* occur in the normal vagina of pregnant or non-pregnant women, may be answered in the affirmative; but with the exception of the gonococcus they are not often seen.[1] Bergholm[2] thus recently examined the vaginal secretion of forty pregnant women, and was unable to obtain organisms pathogenic for animals in a single case. There were no pyogenic staphylococci, no streptococci, and no colon bacilli.

The vaginal secretion has been shown to possess powerful bactericidal properties, so that pathogenic organisms, even when artificially introduced into the vagina, are rapidly killed. Krönig thus found that the bacillus pyocyaneus disappears from the vagina of pregnant women in from ten to thirty hours, the staphylococci in from six to thirty-six hours, and the streptococcus pyogenes within six hours. Important from a practical standpoint is the fact that the bacteria disappeared less rapidly when irrigation of the vagina with water or even antiseptics was employed.

Of animal parasites, the *Trichomonas vaginalis* is occasionally encountered in the vaginal discharge. The organism is identical with the trichomonas found in the feces and the urine. In the United States it is not so common as among the peasant population of Central Europe. As far as is known, the organism is of no pathological significance. From a medicolegal standpoint, however, its presence may not be unimportant, as cases are on record in which trichomonades have been confounded with spermatozoa. In my judgment, however, such a mistake can only occur if the observer is without training in microscopy.

The possible presence of the anguillula aceti in the vaginal discharge has been pointed out by Billings, Miller, and Stiles. Stiles has suggested that it may be introduced into the vagina by injections of vinegar water taken with the object of preventing conception.

[1] Döderlein, Das Scheidensecret, Leipzig, 1892. J. W. Williams, Am. Jour. Obstet., 1898, vol. xxxviii.; Trans. Am. Gyn. Soc., 1898; Am. Jour. Obstet., 1898.

[2] H. Bergholm, Arch. f. Gynäk., 1902, vol. lxvi. Heft 3.

VAGINAL BLENNORRHŒA.

In physiological conditions an increased vaginal secretion is observed during sexual excitement, especially during coitus, just preceding and at the beginning of menstruation, and during pregnancy, when a profuse blennorrhœa is frequently seen, which often assumes a virulent character. The secretion under such conditions readily becomes purulent. When not dependent upon a gonorrhœal infection the secretion is thicker than normal, white, and creamy. At times also the vaginal catarrh observed in pregnancy is complicated with mycosis, when white or yellowish-gray patches may be seen at the orifice of the vagina; the latter may, indeed, even be filled with particles which consist entirely of fungi.

MENSTRUATION.

At the beginning of menstruation, as has been pointed out above, an increase in the amount of vaginal secretion is observed, in which leucocytes, prismatic epithelial cells coming from the uterus, as well as the usual vaginal cells, may be seen upon microscopical examination. Later the secretion becomes sanguineous in character, and finally only epithelial cells, leucocytes, and granular detritus are encountered, the cells usually showing evidence of fatty degeneration. The amount of blood lost at each menstrual period amounts to about 200 grammes in perfectly healthy females.

THE LOCHIA.

The lochia during the first day following parturition are red in color—the *lochia rubra*—and emit the characteristic sanguineous odor. At this time a microscopical examination will reveal an abundance of red corpuscles, some leucocytes, and a variable number of epithelial cells, which are almost exclusively of vaginal origin. On the second and third days the number of red corpuscles diminishes, while the leucocytes increase in number. Still later the diminution in the red and the increase in the white corpuscles become more marked, and the discharge at the same time assumes a grayish or white color, until about the tenth day the red corpuscles have almost entirely disappeared, while the leucocytes and epithelial cells are abundant. Finally, the secretion becomes thicker, mucoid, and milky white in color—the *lochia alba*, which condition may persist for from three to four weeks in nursing-women, and still longer in those who do not nurse, until finally the normal secretion is again established. Numerous bacteria are encountered in the lochia, and it is curious to note that among these pus-organisms are quite constantly present without giving rise to symptoms. When a portion

of the placenta or membranes have been retained the lochia soon give off a fetid odor, and assume a dirty brownish color; the retention of blood-clots alone may also produce this result. In such cases the lochia swarm with bacteria of all kinds.[1]

VULVITIS AND VAGINITIS.

In cases of vulvitis and vaginitis a marked increase is observed in the number of the leucocytes and epithelial cells, the character of the latter depending, essentially of course, upon the portion of the genital tract affected. Red corpuscles are also met with at times; their number generally stands in a direct relation to the intensity of the inflammatory process. In some instances epithelial casts of the entire vagina have been observed, constituting the condition termed *vaginitis exfoliativa*. The condition, however, is rare.

The discharge of large amounts of pure pus through the vagina points to perforation of an abscess of the genital organs or of the neighboring structures into the uterus or the vagina; it is of rare occurrence. Much more common is the discharge of fecal matter or of urine through this channel, indicating the existence of a vagino-rectal or vagino-vesical fistula.

MEMBRANOUS DYSMENORRHŒA.

While ordinarily, during menstruation, shreds of desquamated uterine lining are frequently encountered, it is rare to meet with large pieces or complete casts of the uterus, the elimination of which is usually associated with the symptoms of a severe dysmenorrhœa, constituting the condition spoken of as *membranous dysmenorrhœa*.

CANCER.

While the diagnosis of malignant growth of the uterus is probably never based upon a microscopical examination of the vaginal discharge alone, it may be mentioned that in advanced cases this is possible, as fragments of an epithelioma of the cervix, for example, may frequently be detected upon microscopical examination (Fig. 144). In suspected cases small pieces of tissue should be removed and examined according to usual histological methods.[2]

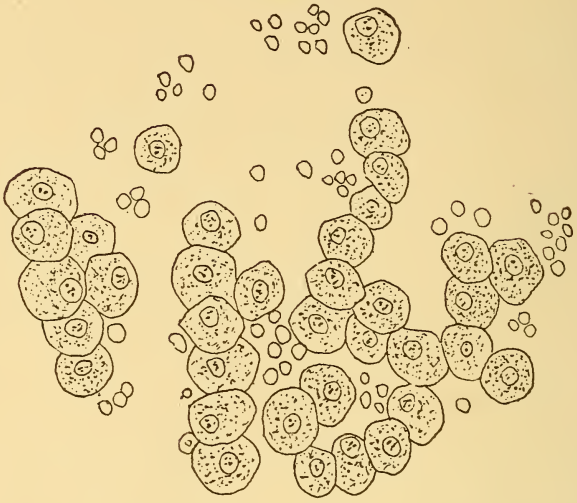
GONORRHŒA.

In suspected cases of gonorrhœa an examination of the vaginal and urethral discharge for the presence of gonococci is important, as it is practically impossible to diagnose this condition positively in any other manner. Care should be taken, however, not to

[1] Döderlein, loc. cit. Thomen, Centralbl. f. d. med. Wiss., 1890, vol. xxviii. p. 537; and Arch. f. Gyn., 1889, vol. xxxvi. p. 231.

[2] T. S. Cullen, Cancer of the Uterus, Appleton & Co., 1900.

FIG. 144.



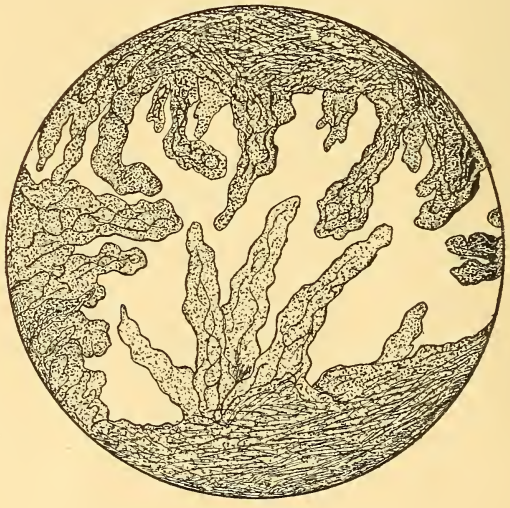
Vaginal secretion from a case of epithelioma of the cervix uteri.

confound the diplococci which may be normally present in the urethra and vagina with gonococci (see chapter on the Urine).

ABORTION.

In cases of abortion it is often possible to discover *chorion villi* in the expelled blood-clots which present the characteristic capillary

FIG. 145.



Chorion villi.

network (Fig. 145), and often manifest signs of advanced fatty degeneration. Important also from a diagnostic point of view is the

FIG. 146.

Decidual cells.

presence of *decidual cells* (Fig. 146), which are characterized by their large size, their round, polygonal, or spindle-like form, and their characteristic nuclei and nucleoli.

CHAPTER XIII.

THE SECRETION OF THE MAMMARY GLANDS.

THE SECRETION OF MILK IN THE NEWLY BORN.

A SECRETION from the mammary glands of the male is observed only in the newly born, if we except those rare cases in which adult males were known to suckle infants. The fluid in question, which may also be obtained from the female infant, is termed "Hexenmilch" (witches' milk) by the Germans. Qualitatively it has the same composition as milk, but may manifest considerable quantitative variations.

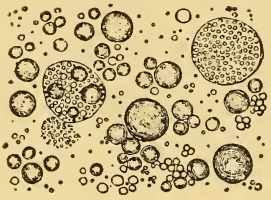
COLOSTRUM.

Aside from those curious instances in which a secretion of milk has been observed in non-pregnant women, mammary activity is essentially connected with the physiological phenomena of pregnancy and parturition. Often as early as the third month a small drop of a serous-looking fluid can be obtained from the nipple by pressure upon the breasts. Immediately after delivery a variable amount of fluid is secreted, which is watery, semi-opaque, mucilaginous, and of a yellowish color. To this secretion, as well as to that observed during pregnancy, the term colostrum has been applied. It is distinguished from true milk by its physical characteristics and by the presence of a greater proportion of sugar and salts. The fluid, moreover, coagulates upon boiling. An idea may be formed of its composition from the appended table:

	4 weeks before birth.		17 days before birth.	9 days before birth.	24 hours after birth.	2 days after birth.
	I.	II.				
Water . . .	945.2	852.0	851.7	858.8	843.0	867.9
Solids . . .	54.8	148.0	148.3	141.2	157.0	132.1
Caseïn . . .	. . .	. . .	. . .	. . .	. . .	21.8
Albumin . .	28.8	69.0	74.8	80.7	. . .	. . .
Fat	7.3	41.3	30.2	23.5	. . .	48.6
Lactose . . .	17.3	39.5	43.7	36.4	. . .	61.0
Salts	4.4	4.4	4.5	5.4	5.1	. . .

Upon microscopical examination fat-droplets, a few leucocytes, some epithelial cells, and so-called *colostrum-corpuscles* are found.

Fig. 147.



Colostrum of a woman in sixth month of pregnancy. (Eye-piece III., obj. 8 A, Reichert.) (v. Jaksch.)

The latter are highly refractive bodies, of irregular size, whose interior is filled with fatty granules (Fig. 147).

Literature.—G. Woodward, Jour. Exper. Med., vol. ii. p. 217.

THE SECRETION OF MILK PROPER, IN THE ADULT FEMALE.

The secretion of milk proper usually begins about the third day following parturition, and may continue for a variable length of time. On the one hand, the amount of milk secreted may be so small as to be insufficient for the needs of the child, so that lactation may have to cease after several days; on the other hand, women are not infrequently seen who nurse their children for two years and even longer. Usually infants are nursed until six or seven teeth have appeared, which period varies with the individual child, averaging about the eleventh month.

HUMAN MILK.

Human milk is of a bluish color, and differs in this respect from the milk of cows. Its reaction is alkaline. The specific gravity may vary between 1.026 and 1.035, one between 1.028 and 1.034 being the most common. The amount of milk secreted in twenty-four hours varies from 500 to 1500 c.c. Microscopically, it is a fairly homogeneous emulsion of fat, and is practically destitute of cellular elements. From the following table an idea may be formed of its chemical composition:

	Biehl.	Gerber.	Christenn.	Pfeiffer.	Pfeiffer.	Mendes de Leon
Water	876.00	891.00	872.40	892.00	890.60	877.90
Solids	124.00	109.00	127.60	108.00	109.40	. . .
Albumin	22.10	17.90	19.00	16.13	17.24	25.30
Fat	38.10	33.00	43.20	32.28	29.15	38.90
Lactose	60.90	53.90	59.80	57.94	59.92	55.40
Salts	2.90	4.20	2.60	1.65	2.09	2.50

Upon comparing this table with the following analysis of cows' milk it will be seen that the latter contains more albumin and less sugar

than human milk. Human milk, moreover, is relatively deficient in mineral matter, and especially in calcium salts and phosphoric acid:

Water	874.2	
Solids	125.8	
Caseïn	28.8	34.5
Albumin	5.3	
Fat	36.6	
Lactose	48.1	
Salts	7.1	

Of inorganic salts human milk contains about 0.7 pro mille of potassium (K_2O), 0.2 of sodium, 0.3 of calcium, 0.06 of magnesium, from 3.52 to 7.21 mgrms. of iron, about 0.4 pro mille of phosphoric acid and 0.4 of chlorine.

The albumins found in milk-plasma are caseïn, lactoglobulin, and lactalbumin. It is claimed by some observers that the caseïn of human milk differs from that obtained from cows' milk. The caseïn-coagula in human milk are not so large and dense as those observed in cows' milk. Human caseïn, moreover, is not so readily precipitated by acids and salts; it does not always coagulate upon the addition of rennet ferment, and while it may be precipitated by the gastric juice, it is readily dissolved by an excess. Although accurate analyses of human caseïn are not available, it is probable that the two forms are not identical (Hammarsten).

The question whether or not normal human milk contains micro-organisms may now be answered in the affirmative. There can be no doubt, however, that the milk as it is secreted by the healthy gland is sterile, but upon passing along the lacteal ducts in the nipple it is always contaminated by the Staphylococcus epidermidis albus (Welch). This micro-organism must be regarded as a constant inhabitant of the skin, and is the only one of the cutaneous bacteria which penetrates the deeper layers of the epidermis and the glandular appendages of the skin. It is thus apparent why this organism is so constantly met with, and is practically the only one found in normal human milk. Exceptionally the Staphylococcus pyogenes aureus is found.

THE MILK IN DISEASE.

The chemistry of the milk in pathological conditions has received little attention. It appears, however, that the milk of women when ill usually contains less fat, and that the proportion of lactose is diminished. In cases of jaundice the presence of bile-pigment and of biliary acids has not been satisfactorily demonstrated. According to Friedjung,[1] a subnormal amount of iron is usually found in the milk when nurslings do not thrive on apparently normal milk. In cases of mammary tumors bloody secretion has been observed in rare cases, the nipple itself being intact.

Microscopically, an admixture of leucocytes is observed in various

[1] G. K. Friedjung, Arch. f. Kinderheilk., vol. xxxi. Hefte 1 u. 2.

diseases of the breast, and especially in cases of abscess. Of pathogenic micro-organisms, streptococci may be found in cases of puerperal fever; more commonly, however, they are absent. The typhoid bacillus has occasionally been seen in cases of typhoid fever, and it is interesting to note that the specific agglutinins of typhoid fever have been found in the milk. Pneumococci have been obtained from the milk of pregnant women affected with lobar pneumonia. The important question whether or not tubercle bacilli are eliminated in the milk in cases of phthisis cannot be definitely answered. In cows such an occurrence is certainly common, even when there is no demonstrable tubercular lesion of the udder. So far as I have been able to ascertain, however, tubercle bacilli have never been found in human milk.[1]

A blue and a red color have been observed in the milk of cows, owing to the presence of the Bacillus pyocyaneus and the Micrococcus prodigiosus, respectively.

A chemical examination of human milk should always be made whenever it is apparent that the nutrition of the baby is below normal. Valuable dietetic suggestions may thus be obtained. In other cases, as when the mother is unwilling or unable to nurse her child beyond a certain period, a knowledge of the composition of her milk will enable the physician to give specific instructions regarding the proper modification of cows' milk. If a wet-nurse is to be employed, her milk should likewise be examined.

Most important is the determination of the specific gravity and of the amount of fat. The former may vary between 1.029 and 1.033. The amount of fat should not be less than 3 per cent.

FIG. 148.

Quevenne's lactodensimeter.

Determination of the Specific Gravity.

The sp. gr. is best determined with the lactodensimeter of Quevenne (Fig. 148). As the instrument is graduated for a temperature of 60° F., it is necessary to correct the sp. gr. whenever the temperature is above or below this point. In the following tables the corrected sp. gr. may be found corresponding to temperatures ranging from 46° to 75° F.:

[1] Escherich, Fortschr. d. Med., 1885, vol. iii. p. 321. Karlinski, Wien. med. Woch., 1888, vol. xxxviii. No. 28. Ott, Prag. med. Woch., 1892, vol. xvii. p. 145. Cohn u. Neumann, Virchow's Archiv, 1880, vol. cxxvi. p. 187.

Corrections for Temperature.

Specific gravity.	Degrees of thermometer (Fahrenheit).									
	46	47	48	49	50	51	52	53	54	55
1020	19.0	19.1	19.1	19.2	19.2	19.3	19.4	19.4	19.5	19.6
1021	20.0	20.0	20.1	20.2	20.2	20.3	20.3	20.4	20.5	20.6
1022	21.0	21.0	21.1	21.2	21.2	21.3	21.3	21.4	21.5	21.6
1023	22.0	22.0	22.1	22.2	22.2	22.3	22.3	22.4	22.5	22.6
1024	22.9	23.0	23.1	23.2	23.2	23.3	23.3	23.4	23.5	23.6
1025	23.9	24.0	24.0	24.1	24.1	24.2	24.3	24.4	24.5	24.6
1026	24.9	24.9	25.0	25.1	25.1	25.2	25.2	25.3	25.4	25.5
1027	25.9	25.9	26.0	26.1	26.1	26.2	26.2	26.3	26.4	26.5
1028	26.8	26.8	26.9	27.0	27.0	27.1	27.2	27.3	27.4	27.5
1029	27.8	27.8	27.9	28.0	28.0	28.1	28.2	28.3	28.4	28.5
1030	28.7	28.7	28.8	28.9	29.0	29.1	29.1	29.2	29.4	29.4
1031	29.6	29.6	29.7	29.8	29.9	30.0	30.1	30.2	30.3	30.4
1032	30.5	30.5	30.6	30.7	30.9	31.0	31.1	31.2	31.3	31.4
1033	31.4	31.4	31.5	31.6	31.8	31.9	32.0	32.1	32.3	32.4
1034	32.3	32.3	32.4	32.5	32.7	32 9	33.0	33.1	33.2	33.3
1035	33.1	33.2	33.4	33.5	33.6	33.8	33.9	34.0	34.2	34.3

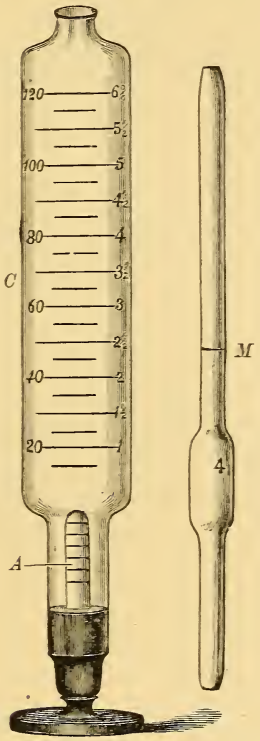
Specific gravity.	Degrees of thermometer (Fahrenheit).									
	56	57	58	59	60	61	62	63	64	65
1020	19.7	19.8	19.9	19.9	20.0	20.1	20.2	20.2	20.3	20.4
1021	20.7	20.8	20.9	20.9	21.0	21.1	21.2	21.3	21.4	21.5
1022	21.7	21.8	21.9	21.9	22.0	22.1	22.2	22.3	22.4	22.5
1023	22.7	22.8	22.8	22.9	23.0	23.1	23.2	23.3	23.4	23.5
1024	23.6	23.7	23.8	23.9	24.0	24.1	24.2	24.3	24.4	24.5
1025	24.6	24.7	24.8	24.9	25.0	25.1	25.2	25.3	25.4	25.5
1026	25.6	25.7	25.8	25.9	26.0	26.1	26.2	26.3	26.5	26.6
1027	26.6	26.7	26.8	26.9	27.0	27.1	27.3	27.4	27.5	27.6
1028	27.6	27.7	27.8	27.9	28.0	28.1	28.3	28.4	28.5	28.6
1029	28.6	28.7	28.8	28.9	29.0	29.1	29.3	29.4	29.5	29.6
1030	29.6	29.7	29.8	29.9	30.0	30.1	30.3	30.4	30.5	30.7
1031	30.5	30.6	30.8	30.9	31.0	31.2	31.3	31.4	31.5	31.7
1032	31.5	31.6	31.7	31.9	32.0	32.2	32.3	32.5	32.6	32.7
1033	32.5	32.6	32.7	32.9	33.0	33.2	33.3	33.5	33.6	33.8
1034	33.5	33.6	33.7	33.9	34.0	34.2	34.3	34.5	34.6	34.8
1035	34.5	34.6	34.7	34.9	35.0	35.2	35.3	35.5	35.6	35.8

Specific gravity.	Degrees of thermometer (Fahrenheit).									
	66	67	68	69	70	71	72	73	74	75
1020	20.5	20.6	20.7	20.0	21.0	21.1	21.2	21.3	21.5	21.6
1021	21.6	21.7	21.8	22.0	22.1	22.2	22.3	22.4	22.5	22.6
1022	22.6	22.7	22.8	23.0	23.1	23.2	23.3	23.4	23.5	23.7
1023	23.6	23.7	23.8	24.0	24.1	24.2	24.3	24.4	24.6	24.7
1024	24.6	24.7	24.9	25.0	25.1	25.2	25.3	25.5	25.6	25.7
1025	25.6	25.7	25.9	26.0	26.1	26.2	26.4	26.5	26.6	26.8
1026	26.7	26.8	27.0	27.1	27.2	27.3	27.4	27.5	27.7	27.8
1027	27.7	27.8	28.0	28.1	28.2	28.3	28.4	28.6	28.7	28.9
1028	28.7	28.8	29.0	29.1	29.2	29.4	29.5	29.7	29.8	29.9
1029	29.8	29.9	30.1	30.2	30.3	30.4	30.5	30.7	30.9	31.0
1030	30.8	30.9	31.1	31.2	31.3	31.5	31.6	31.8	31.9	32.1
1031	31.8	32.0	32.2	32.2	32.4	32.5	32.6	32.8	33.0	33.1
1032	32.9	33.0	33.2	33.3	33.4	33.6	33.7	33.9	34.0	34.2
1033	33.9	34.0	34.2	34.3	34.5	34.6	34.7	34.9	35.1	35.3
1034	34.9	35.0	35.2	35.3	35.5	35.6	35.8	36.0	36.1	36.3
1035	35.9	36.1	36.2	36.4	36.5	36.7	36.8	37.0	37.2	37.3

Estimation of the Fat.

The estimation of the fat is most conveniently made by means of the lactoscope of Feser, shown in Fig. 149. Milk is drawn into

Fig. 149.



Feser's lactoscope.

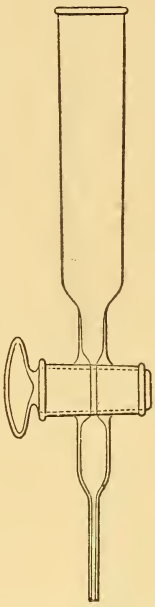
the pipette up to the mark *M*, when it is emptied into the cylinder *C*. The pipette is then rinsed with water and the washings added to the milk. While shaking, water is added until the black lines upon the milk-colored glass plug *A* can just be discerned. The figure upon the right of the scale at the level reached by the mixture indicates the percentage-amount of fat, while the number upon the left indicates in cubic centimeters the amount of water that has been added.

Estimation of the Proteids.

Woodward's Method.—Two "milk-burettes" (see Fig. 150), each containing 5 c.c. of milk, are kept at a temperature of from 37° to 40° C. for from eighteen to twenty-four hours. At the end of

this time the milk has separated into two layers, viz., an upper layer of viscid yellow fat, and a lower layer of fluid milk, which is quite opaque above and almost translucent below. Clinging to the sides of the tube, and especially at the bottom, a granular precipitate will be seen. The burettes are then cooled, when the milk-serum is

FIG. 150.



Woodward's milk-burette.

withdrawn into two tubes graduated to 15 c.c., and treated with Esbach's reagent to the 15 c.c. mark. The mixture in each tube is thoroughly stirred with a glass rod and then centrifugated to a constant reading.

Woodward [1] has checked his analyses by Kjeldahl's method, and has obtained satisfactory results.

[1] G. Woodward, "A Clinical Method for the Estimation of Breast-milk Proteids," Phila. Med. Jour., 1898, p. 956.

INDEX.

ABORTION, vaginal discharge in, 670
Absorption, rate of, in the stomach, 268
Acetic acid, 255
fermentation, 254
tests for, 255
Acetonæmia, 54
Acetone in the blood, 54
in the gastric contents, 258
in the urine, 568
quantitative estimation of, 571
tests for, 569
Acetonuria, 568
Acholic stools, 287, 295
Achroödextrin, 199, 245
Acid, acetic, 255, 281
benzoic, 457
butyric, 254, 281
carbolic, 279, 566
diacetic, 573
diazo-benzene-sulphonic, 559
formic, 281
glucuronic, 533
hippuric, 457
homogentisinic, 555
hydrochloric, 217
lactic, 245
oxalic, 466
oxaluric, 466
oxybutyric, 574
phosphoric, 395
propionic, 281
succinic, 659
sulphuric, 404
tauro-carbaminic, 411
uric, 442
uroleucinic, 555
valerianic, 282
Acids, organic, in the gastric contents, 253
Acquisto's fluid, 142
Actinomyces hominis, 203, 362, 627
Actinomycosis, 203, 362
Adenin in the urine, 455
Afanassiew's reagent, 113
Agglutinins, 170
a-granulation of Ehrlich, 79
Albumin, aceto-soluble, 485
in the feces, 333
in the urine, 472
quantitative estimation of, 499
special test for serum-albumin, 498
for serum-globulin, 502
Albumin, tests for, 492
boiling, 496
nitric acid, 493
picric acid, 498
potassium ferrocyanide, 497
Spiegler's, 498
trichloracetic acid, 497
Albuminimeter, 500
Albuminous expectoration, 366
Albuminuria, 472
accidental, 484
colliquative, 479
constitutional, 475
cyclic, 474
Da Costa's, 476
digestive, 483
febrile, 477
functional, 474, 476
hæmatogenous, 475, 481
in organic diseases of the kidneys, 477, 484
intermittent, 474
mixed, 484
neurotic, 482
orthostatic, 474
physiological, 472
postural, 474
referable to circulatory disturbances, 480
to impeded outflow of urine, 481
renal, 477, 484
toxic, 482
transitory, 474
Albumoses in the blood, 44
in the feces, 333
in the gastric contents, 244
in the urine, 486
tests for, 502
Albumosuria, 486
digestive, 487
enterogenic, 486
hæmatogenic, 486
hepatogenic, 486
histogenic, 486
mixed, 487
pyogenic, 486
renal, 486
vesical, 486
Alkalimeter, Engel's, 25
Alkaline stools, 278, 286
urine, 381
Alkalinity of the blood, 20
distribution of, 139

Alkalinity of the blood, estimation of, 21
Alkapton in the urine, 555
Alkaptonuria, 555
Alloxur bases in the urine, 442, 455
estimation of, 456
Almén's solution, 518
Alveolar epithelium, 349
Ammonia in the blood, 47
in the gastric contents, 257
in the urine, 439
estimation of, 441
Ammoniacal fermentation, 382
Ammonio-magnesium phosphate, 591
Ammonium urate, 601
Amœba coli, 299
in the feces, 299
in the sputum, 353
Amœbæ in the urine, 629
Amœbinæ in the feces, 298
Amphistomum hominis, 315
Amphoteric urine, 381
Amyloid corpuscles in the semen, 663
Anachlorhydria, 224
Anacidity, hysterical, 224
Anadeny of the stomach, 224
Anæmic degeneration of the red corpuscles, 63
Anchylostomiasis, 317
Anchylostomum duodenale, 317
Anguillula aceti, 629, 667
intestinalis, 320, 321
stercoralis, 320, 321
Anguilluliasis, 197, 320
Anilin dyes, classification of, 119
water, gentian-violet, 207
Animal gum in the urine, 533
parasites in the blood, 182
in the feces, 297
in the sputum, 352
in the urine, 628
Annelides, 315
Anthracosis of the lungs, 365
Anthrax, bacillus of, 177
Arabinose in urine, 532
Arnold's test for diacetic acid, 573
Ascarides in the feces, 315
in the urine, 629
Ascaris lumbricoides, 315
maritima, 316
mystax, 316
Asiatic cholera, bacillus of, 323
Asthma, bronchial, Charcot-Leyden crystals in, 364
Azoöspermatism, 663

BACILLI of Booker, 325
Bacillus acidophilus, 329
coli communis, 328
dysenteriæ, 331
lactis aërogenes 328
melitensis, 180
of anthrax, 177
Bacillus of cholera Asiatica, 323
of diphtheria, 206
of dysentery, 331
of Finkler and Prior, 325
of glanders, 178
of influenza, 178, 360
of leprosy, 356
of Le Sage, 325
of Malta fever, 180
of Moro, 329
of Oppler and Boas, 264
of paratyphoid fever, 170
of plague, 181, 362
of Shiga, 331
of tuberculosis, in the blood, 177
in the feces, 328
in the gastric contents, 264
in the meningeal fluid, 654
in the milk, 675
in the mouth, 203
in the nasal discharge, 336
in the sputum, 354
in the urine, 626
methods of staining, 357
of typhoid fever, in the blood, 165
in the feces, 325
in the sputum, 362
in the urine, 625
of whooping-cough, 361
of yellow fever, 180
pestis, 181, 362
pyocyaneus, 329
smegma, 356, 361
Bacteria in blood, 165
in exudates, 635
in feces, 276, 323
in gastric contents, 264
in milk, 675
in mouth, 203
in nasal secretion, 336
in pus, 642
in sputum, 354
in urine, 626
in vagina, 667
Bacterial decomposition of the urine, 382
Bacteriuria, 623
idiopathic, 627
Bacterium lactis aërogenes, 328
Balantidium coli, 304
Bang's test for albumoses, 503
for urobilin, 503
Barfoed's reagent, 245
Barfurth's reagent, 138
Basic anilin dyes, 119
double stain, 136
phosphate of magnesium, 593
Basophilic leucocytes in the blood, 80
in the sputum, 348
perinuclear granules, 78
Baumann and v. Udranszky's method of isolating diamins, 583
Beckmann's apparatus, 164
Bence Jones' albumin, 487

Bence Jones' albumin, tests for, 504
Benzoic acid in the urine, 457
Benzopurpurin test for hydrochloric acid, 228
Bile-pigment in the blood, 53
in the feces, 333
in the gastric contents, 261
in the urine, 548
tests for, 550
Gmelin's, 550
Huppert's, 550
Rosenbach's, 550
Smith's, 550
Bilharzia hæmatobia, 195
eggs in the urine, 629
Bilharziasis, 195
Biliary acids in the blood, 53
in the feces, 283
in the urine, 550
tests for, 283
concretions, 291
analysis of, 292
Bilirubin, 548, 599
Biuret test, 423
Blood, 17
acetone in, 54
albumins in, 27, 43
albumoses in, 43
alkalinity of, 20
ammonia in, 47
bacteriology of, 165
biliary constituents in, 53
carbohydrates in, 44
cellulose in, 47
chemical examination of, 27
coagulation of, 28
color of, 17
color-index, 57
crisis, 69
drying and staining of, 115
dust, 114
examination, technique of, 114
fat in, 50
fatty acids in, 50
fibrin in, 27, 43
gases in, 31
general characteristics of, 17
chemistry of, 27
glycogen in, 46
hæmokonia of, 114
in the feces, 274, 287, 296
in the gastric contents, 261
in the sputum, 340, 348
in the urine, 413, 610
kryoscopy of, 163
lactic acid in, 52
leucocytes of, 17, 71
medico-legal test for, 39
methods of staining, 123
microscopical examination of, 54
nucleated corpuscles in, 67
odor of, 18
parasites in, 165
Blood, parasitology of, 165
peptone in, 44
pigments of, 32
plaques, 17, 112
proteids in, 43
protozoa in, 182
reaction of, 21
red corpuscles, 17, 54
solids of, 20
specific gravity of, 18
staining of, 119
sugar in, 44
tests for, 39, 507
Donogany's, 262
guaiacum, 507
Heller's, 507
Korczynski and Jaworski's, 288
Müller and Weber's, 261
urea in, 47
uric acid in, 48
xanthin-bases in, 49
Blood-corpuscles, red, 54
anæmic degeneration of, 62
behavior toward anilin dyes, 62
crenation of, 56
granular degeneration of, 65
enumeration of, 139
money roll formation, 56
nucleated, 67
polychromatophilia of, 62
variations in color, 56
in form, 55
in number, 58
in size, 54
white. *See* Leucocytes.
Blood-crisis, 69
Blood-iron, 158
Blood-pigments, 32
Blood-plasma, 17, 30
Blood-plates, 112
Blood-serum, 28
Blood-shadows, 610
Boas' bulbed stomach-tube, 213
method for estimating lactic acid, 251
test for hydrochloric acid, 227
for lactic acid, 250
Boas-Oppler bacillus, 264
Bodo urinarius, 620
Boiling test for albumin, 496
Bothriocephalus latus, 311
Böttcher's crystals, 364, 661
Böttger's test for sugar, 518
Bremer's diabetic blood test, 63
urine test, 530
Brodie and Russell's method of enumerating the plaques, 147
Browning's spectroscope, 42
Bubonic plague, bacillus of, 181
Buccal secretion (*see* Saliva), 198
Butyric acid fermentation, 254
in the feces, 281
in the gastric contents, 254
test for, 254

CABOT'S ring bodies, 67
Cadaverin, 582
Cahn-Mehring's method of estimating fatty acids, 255
Calcium carbonate, crystals of, 601
oxalate, crystals of, 292, 590
phosphate, crystals of, 600
sulphate, crystals of, 593
Calomel stools, 271
Carbohydrates, digestion of, 242
in the blood, 44
in the feces, 333
in the urine, 503
tests for, 243
Carbol-fuchsin, 357
Carbolic acid, estimation of, 567
test for, 280, 567
Carbolo-chloride of iron test for lactic acid, 248
Carbon dioxide hæmoglobin, 38
monoxide hæmoglobin, 37
Caries of the teeth, 198
Caseïn, digestion of, 242
in the milk, 678
test for, Leiner's, 295
Casts, classification of, 612
examination of, 613
fatty, 616
fibrinous, 342
formation of, 618
granular, 615
hyaline, 614
pus, 615
significance of, 619
staining of, 614
urinary, 612
waxy, 616
Cause's method of estimating sugar, 524
Cellulose in the blood, 47
Cenomonadina, 301
Cercomonas intestinalis, 301
Cerebrospinal fluid, 649
amount of, 650
appearance of, 650
chemical composition of, 652
cytodiagnosis of, 653
microscopical examination of, 653
reaction of, 652
specific gravity of, 651
toxicity of, 656
Cestodes, 232
Chalicosis, 366
Charcot-Leyden crystals, in the feces, 275
in the nasal discharge, 337
in the sputum, 345, 364
Chemical examination of blood, 27
of cystic fluids, 657
of exudates, 638
of feces, 278, 333
of gastric juice, 216
of milk, 677
of pus, 640
Chemical examination of saliva, 198
of semen, 661
of sputum, 366
of transudates, 633
of urine, 385
Chenzinsky-Plehn stain, 137
Chlorides in the urine, 387
estimation of, 390
according to Neubauer and Salkowski, 395
according to Salkowski and Volhard, 390
direct method, 394
test for, 390
Chloroform-benzol mixture, 19
Cholæmia, 53
Cholera Asiatica, bacillus of, 323
infantum, bacillus of, 331
Cholesterin in the blood, 31
in the feces, 282
in the sputum, 365
in the urine, 551
isolation of, from the feces, 282
test for, 283
Choluria, 549
Chorion villi, 670
Chromogens in the urine, 537
Chyluria, 579, 600
Chymosin, 239
estimation of, 241
test for, 240
Chymosinogen, 239
estimation of, 241
test for, 240
Ciliated epithelium in cysts, 658
in the sputum, 348
Cladothrix, 362
Coagulation of the blood, 28
Coagulometer, Wright's, 29
Coating of the tongue, 204
Coffin-lid crystals, 591
Colloid concretions in ovarian cysts, 658
Color index of the blood, 57
Colostrum, 672
Comma bacillus, 323
Concretions, biliary, 291
fecal, 292
intestinal, 291
pulmonary, 346
Congo-red test for free acids, 225
Conjugate glucuronates, 533
sulphates, 336, 555, 566
Copper test for uric acid, 449
Coproliths, 292
Corpora amylacea, 663
Crotonic acid, 576
Crystals, ammonio-magnesium phosphate, 591
bilirubin, 599
calcium carbonate, 601
oxalate, 365
phosphate, 592, 593
sulphate, 593

Crystals, Charcot-Leyden, 297
cholesterin, 365
cystin, 594
fatty acids, 365
hæmatoidin, 599
hæmin, 39
hippuric acid, 593
indigo, 601
in the feces, 274, 297
leucin, 365
leucocytic, 364
magnesium phosphate, 593, 600
monocalcium phosphate, 592
neutral calcium phosphate, 593
phenyl-glucosazon, 520
phosphate of spermin, 661
Teichmann, 39
triple phosphate, 365
tyrosin, 365
urate of ammonium, 601
uric acid, 588
xanthin, 598
Curschmann's spirals, 344
Cylinders, mucous, in the feces, 290
in the urine, 618
urinary, 612
Cylindroids, 618
Cylindruria, 612
Cystein, 411
Cysticercus cellulosæ, 308
Cystin, 412, 594
Cystinuria, 412, 582, 594
Cysts, colloid, 658
contents of, 657
dermoid, 658
fibrocystic, 658
hydatid, 659
ovarian, 659
pancreatic, 660
parovarian, 658
Cytodiagnosis in cerebrospinal fluid, 653
in pleural effusions, 635

DALAND'S hæmatokrit, 147
Dare's hæmoalkalimeter, 25
hæmoglobinometer, 151
method of estimating the alkalinity of the blood, 25
Decidual cells, 671
Deetjen's agar, 112
δ-granulation of Ehrlich, 80
Dennigè's test for acetone, 54, 570
Dermoid cysts, 658
Dextrin in the urine, 532
Dextrose in the urine. *See* Glucose.
Diabetes, 515
alternans, 447
Bremer's blood test in, 63
urine test in, 530
hepatogenic, 516
Hirschfeld's form of, 517
Diabetes, insipidus, 374, 534
myogenic, 516
phosphatic, 398
Williamson's blood test in, 46
Diabetic chromatophilia, 63
Diacetic acid in the urine, 573
tests for, 573
Diaceturia, 573
Diamins in the feces, 334
in the urine, 582
isolation of, 583
Diarrhœa, 285
Diathesis, oxalic acid, 466
uric acid, 446
Diazo-reaction (*see* Ehrlich's reaction), 559
Differential density method of estimating sugar, 526
Digestion, gastric, 241
of albumins, 241
of albuminoids, 242
of carbohydrates, 242
of milk, 242
of proteids, 242
products of, 241
Dimethylamidoazobenzol test, 226
Diphtheria, 206
Diplococcus meningitidis intracellularis, 654
pneumoniæ, 360
in the blood, 171
Dipylidium caninum, 309
Distoma Buskii, 314
capense, 195
conjunctum, 315
hæmatobium, 195, 315, 354
hepaticum, 313
heterophyes, 315
lanceolatum, 314
pulmonale, 315, 353
rhatonisi, 314
sibiricum, 314
spatulatum, 314
Distomiasis, 195
Donné's pus test, 607
Donogany's blood test, 262, 507
Doremus' ureometer, 428
Drugs, effect of, on the color of the stools, 271
Drysdale's corpuscles, 659
Dunlop's method of estimating oxalic acid, 471
Dust-particles of Müller, 114
Dyes, 119

EARTHY phosphates, 395
Eberth's bacillus, 325
Echinococcus, 345, 352
membranes in the sputum, 345
polymorphus, 352
ε-granulation of Ehrlich, 77
Ehrlich's dahlia, 136

Ehrlich's diazo-reaction, 559
dimethylamidobenzaldehyde reaction, 564
eosin-methylene-blue methylal, 133
hæmatoxylin-eosin, 138
hæmoglobinæmic Innenkörper, 67
methyl-green-fuchsin stain, 136
neutral red, 645
tri-acid stain, 124
tri-glycerin mixture, 135
Einhorn's bucket, 215
saccharimeter, 519, 526
Elastic tissue in the sputum, 342, 350
stain for, 351
Elsner's method, 327
Engel's alkalimeter, 25
method of estimating the alkalinity of the blood, 24
Enterogenic albumosuria, 486
Enteroliths, 292
Eosin, staining with, 135
Eosin-methylal and methylene-blue, 133
Eosinate of methylene-blue, staining with, 123
Eosinophilia, 100
Eosinophilic leucocytes in the blood, 79
in the sputum, 347
Epithelial cells, alveolar, 349
ciliated, 349
in the buccal secretion, 200
in the feces, 274, 295
in the gastric contents, 201
in the sputum, 348
in the urine, 602
in the vaginal secretions, 666
Eructatio nervosa, 258
Erythrodextrin, 242
test for, 242
Erythrosin, acid, staining with, 139
Esbach's albuminimeter, 500
method of estimating albumin, 500
reagent, 500
Escherich's stain, 329
Ethyl sulphide, 411
Euchlorhydria, 224
Eustrongylus gigas, 629
Ewald's modification of Mohr's test for hydrochloric acid, 228
Ewing's stain, 132
Extractives in the blood, 31
Exudates, 634
bacteriological examination of, 639
chemistry of, 638
chyloid, 647
chylous, 647
hemorrhagic, 634
in cancer, 636
in tuberculosis, 635
purulent (*see* Pus), 640
putrid, 649
serous, 634

FARRANT'S solution, 614
Fat in the blood, 50
in the milk, estimation, 677
in the urine, 578, 599
Fatty acids, clinical significance of, 253
estimation of, 255
formation of, 253
in pus, 641
in the blood, 50
in the feces, 280
in the gastric contents, 253
in the sputum, 363
in the urine, 577
tests for, 253
casts, 616
Febrile acetonuria, 468
albuminuria, 477
urobilin, 552
Fecal matter in the urine, 630
vomiting, 262
Feces, 270
alimentary detritus in, 272, 289
amount of, 270, 286
annelides in, 315
biliary acids in, 282
concretions in, 227
blood in, 287, 296
chemistry of, 278, 333
cholesterin in, 282
color of, 271, 287
composition of, 210
concretions in, 291
consistence of, 271, 285
crystals in, 274, 297
epithelial cells in, 274, 295
examination of normal, 207
fatty acids in, 280
flagellata in, 300
foreign bodies in, 272
form of, 271, 285
gases in, 279
general characteristics of, 270, 285
indol in, 279
leucocytes in, 274, 296
macroscopical constituents of, 272, 289
microscopical constituents of, 272, 293
mucus in, 290, 296
number of stools, 270, 285
odor of, 271, 286
parasites in, 275, 297, 323
animal, 297
vegetable, 275, 323
pathology of, 285
phenol in, 279
pigments in, 283
protozoa in, 298
ptomaïns in, 334
reaction of, 278, 286
skatol in, 279
technique in examination of, 293
trematodes in, 313

Feces, vermes in, 305
Fehling's method of estimating sugar, 523
solution, 518
test for sugar, 517
Ferment, milk-curdling, 239
of saliva, 199
Fermentation test for sugar, 519
Schmidt's fecal, 293
Ferments in the gastric juice, 236
in the urine, 579
Ferrocyanide test for albumin, 497
Ferrometer, Jolles', 158
Feser's lactoscope, 677
Fibrin, 28, 43
estimation of, 43
ferment, 28
in the blood, 28
in the urine, 490
test for, 508
Fibrinogen, 28
Fibrinoglobulin, 28
Fibrinous casts, 342
coagula in the sputum, 342
in the urine. *See* Chyluria.
Filaria Bancrofti, 192
demarquaii, 192
diurna, 192
Mansoni, 192
nocturna, 192
ozzardi, 192
perstans, 195
sanguinis hominis, 192
Wuchereri, 192
Filariasis, 192
Finkler-Prior bacillus, 325
Flagellata, 300
Fleischl's hæmometer, 153
Florence's test for semen, 664
Folin's method of estimating ammonia, 441
kreatinin, 464
urea, 434
uric acid, 450
Foreign bodies in the feces, 272
in the sputum, 346
in the urine, 630
Formic acid, detection of, 281
Freund's method of determining acidity of urine, 384
Furfurol test for bile acids, 283
Futcher's stain, 137

GABETT'S staining method, 357
Galacturia, 599
Gall-stones, analysis of, 292
in the feces, 291
Garrod's test for hæmatoporphyrin in the urine, 547
for homogentisinic acid, 558
for uric acid in the blood, 49
Gases in the blood, 31
in the feces, 279
in the gastric contents, 256
Gases in the urine, 580
Gastric contents, examination of (*see* Gastric juice), 210
digestion of albuminoids, 242
of carbohydrates, 242
of native albumins, 241
of proteids, 242
products of, 241
analysis of, 244
juice, 210
acetic acid in, 253
acetone in, 258
acidity of, 217, 219
amount of, 215
antiseptic properties of, 222
blood in, 261
butyric acid in, 253
cause of acidity of, 217
chemical composition of, 216
examination of, 216
chymosin in, 239
chymosinogen in, 239
fatty acids in, 253
ferments in, 236
free acid in, 225
gases in, 256
general characteristics of, 215
hydrochloric acid in, 217, 224
hyperacidity of, 221
hypersecretion of, 221
lactic acid in, 245
methods of obtaining, 213
microscopical examination of, 264
milk-curdling ferment of, 239
organic acids in, 253
pepsin in, 236
pepsinogen in, 236
ptomains and toxalbumins in, 258
secretion of, 210
zymogens in, 236
Gastrosuccorrhœa mucosa, 260
Gerhardt's test for diacetic acid, 573
for urobilin, 553
Giemsa's stain, 129
Gigantoblasts (*see* Megaloblasts), 69
Glanders, bacillus of, 178
Glandular fever, 206
Glaser's method of estimating neutral sulphur, 413
Globin, 32
Glucose, 508
in the blood, 44
estimation of, 45
in the urine, 508
quantitative estimation of, 522
tests for, 517
Glucosuria, 508
digestive, 508
e saccharo, 512
ex amylo, 512
persistent, 514

Glucosuria, transitory, 513
Glucosuric acid, 555
Glucuronic acid in the blood, 44
in the urine, 533
Glycogen in the blood, 46
test for, 138
in the sputum, 366
Gmelin's reaction, 550
Gonococcus in the blood, 176
in the mouth, 203
in the urine, 627
staining of, 645
in urethral discharge, 644
Neisser's, 644
Gonorrhœal pus, 644
stomatitis, 203
threads in the urine, 608, 646
Gowers' hæmoglobinometer, 157
Gram's method of staining, 207
Granular degeneration, 65
γ-granulation of Ehrlich, 80
Grape-sugar. *See* Glucose.
Green's ureometer, 432
Gregarina, 305
Grethe's method of staining tubercle bacilli in the urine, 626
Guaiacum test for blood, 507
Guanin in the urine, 507
Gum, animal, 533
Gunning's mixture, 435
Günzburg's packages, 268
reagent, 226
Gynæcophorus, 195

HÆMATEMESIS, 262
Hæmatin, 38
Hæmatinuria, 489, 545
Hæmatoblasts, 112
Hæmatoidin in the blood, 40
in the sputum, 364
in the urine, 599
Hæmatokrit, 147
Hæmatoporphyrin in the blood, 41
in the feces, 284
in the urine, 546
tests for, 547
Hæmatoporphyrinuria, 546
Hæmaturia, 490, 610
Hæmin (*see* Teichmann's crystals), 39
Hæmo-alkalimeter, Dare's, 26
Hæmochromogen, 32
Hæmocytometer of Thoma-Zeiss, 139
Hæmoglobin, 17, 32
carbon dioxide, 38
monoxide, 37
estimation of, with Dare's instrument, 151
with Fleischl's hæmometer, 153
with Gowers' hæmoglobinometer, 157
with Oliver's instrument, 155
with Talquist's method, 158
hydrogen sulphide, 38
Hæmoglobin, nitric oxide, 38
tests for, 39, 506
Hæmoglobinæmia, 36
Hæmoglobinometer, 150
Hæmoglobinuria, 36, 489
Hæmokonia, 114
Hæmometers, 150
Hæmospermia, 663
Halitus sanguinis, 18
Hammerschlag's method of determining the specific gravity of blood, 19
of estimating pepsin, 238
Haycraft's method of estimating uric acid, 452
Hayem's fluid, 141
Heart-disease cells, 350
Hehner-Seemann's method of estimating organic acids, 255
Heller's test for albumin, 493
for blood, 507
Hepatogenic icterus, 549
Heteroxanthin in the urine, 455
Hippuric acid in the urine, 457, 593
estimation of, 459
properties of, 458
test for, 458
Histon in the urine, 492
test for, 508
Hoffmann's test for tyrosin, 597
Hofmeister's method of estimating hippuric acid, 460
test for leucin, 598
Homogentisinic acid in the urine, 555
estimation of, 558
isolation of, 557
Hopkins' method of estimating uric acid, 449
Hüfner's ureometer, 432
Huppert's test for bile-pigment, 550
Hydatid cysts, 659
echinococcus membranes and hooklets in, 659
sodium chloride in, 659
succinic acid in, 659
disease, 352
Hydrobilirubin, 284
Hydrocele agar, 646
fluid, 633
cholesterin in, 633
Hydrochinon in the urine, 567
Hydrochloric acid in the gastric juice, 217
amount of, 224
combined, 229
estimation of, according to Leo, 235
according to Martius and Lüttke, 232
according to Sahli, 232
according to Töpfer, 230
free, 224
significance of, 222
source of, 221

Hydrochloric acid, tests for, 225
Hydrogen sulphide, in the gastric contents, 257
tests for, 257
in the urine, 580
Hydronephrosis, 659
Hydrothionuria, 342, 507, 580, 627
Hymenolepis, 308
Hypalbuminosis, 43
Hypeosinophilia, 104
Hyperalbuminosis, 43
Hyperchlorhydria, 225
Hyperinosis, 43
Hyperisotonia, 31
Hyperleucocytosis, 85
polynuclear eosinophilic, 100
neutrophilic, 86
Hypersecretio acida et continua, 221, 225
Hypersecretion, 216, 221
Hypinosis, 43
Hypobromite method of estimating urea, 426
solution, 426
Hypochlorhydria, 224
Hypoleucocytosis, 85
polynuclear eosinophilic, 104
neutrophilic, 96
Hypoxanthin in the urine, 455

ICTERUS, 549
hæmatogenic, 549
hepatogenic, 549
neonatorum, 549
urobilin, 552
Idiopathic bacteriuria, 627
oxaluria, 468
Ilasvay's reagent, 200
Indican in the urine, 537
estimation of, 541
tests for, 540
Indicanuria, 537
Indigo-blue in the urine, 559, 581, 601
Indigo-red in the urine, 543
Indigosuria, 559, 581, 601
Indol in the feces, 279
tests for, 280
Indoxyl, 537
sulphate (*see* Indican), 537
Influenza, bacillus of, 178
Infusoria in pus, 643
in the feces, 297
in the urine, 629
in vaginal discharges, 667
Inosit in the urine, 534
Insects in the feces, 323
Intermittent albuminuria, 474
Intestinal concretions, 292
sand, 292
Iodoform-test for lactic acid, 250
Iodophilia, 83
demonstration of, 138
Iodospermin, 664
Iron in blood, 36
Isomastigoda, 300
Isotonia, 30

JAFFE'S test for indican, 541
Jaundice (*see* Icterus), 549
Jenner's stain, 123
Jolles' ferrometer, 158
Justus' syphilitic blood test, 35

KELLING'S test for lactic acid, 249
Kjeldahl's method, 435
Knapp's method of estimating sugar, 525
Koch's stain, 132
Koplik's bacillus, 361
Korczynski and Jaworski's test, 288
Krabbea grandis, 313
Kreatin, 460
properties of, 461
Kreatinin, 460
estimation of, 462
properties of, 461
test for, 462
Kreatinin-zinc chloride, 462
Kryoscopy of the blood, 163
of the urine, 585

LACMOID paper, preparation of, 24
Lactic acid, 245
clinical significance of, 245
estimation of, 251
fermentation, 248
in the blood, 52
in the gastric contents, 245
in the urine, 576
mode of formation, 245
tests for, 248
Boas', 250
Kelling's, 249
Strauss', 249
Uffelmann's, 248
Lactodensimeter of Quevenne, 675
Lactoscope of Feser, 677
Lactose in the urine, 531
Lævulose in the urine, 531
Laiose in the urine, 532
Landois' estimation of the alkalinity of the blood, 21
Large mononuclear leucocytes, 75
clinical variations of, 107
Laveran's organism, 182
Laverania malariæ, 187
Lecithin in the blood, 31
Legal's test for acetone, 569
Leiner's test for caseïn, 295
Leishman's stain, 130
Leo's method of estimating hydrochloric acid, 235
Leprosy, bacillus of, 356
Leptothrix buccalis, 200
Leube's test of motor power of stomach, 267
Leucin, 595
Leucocytes, 17, 71

Leucocytes, basophilic, 80
degenerative changes, 77, 641
differential enumeration, 146
differentiation according to their behavior toward anilin dyes, 73
Ehrlich's granulations in, 77, 79, 80
enumeration of, 143
eosinophilic, 79
estimation of the number of, 143
general differentiation of the various forms, 71
indirect enumeration of, 145
in the blood, 17, 71
in the exudates, 635
in the feces, 274, 296
in the sputum, 346
in the urine, 605
irritation forms, 83
large mononuclear, 75
lymphogenic, 73
myelocytes, 81
neutrophilic, 77
oxyphilic, 79
polymorphonuclear, 72, 77
polynuclear, 72, 77
pseudolymphocytes, 83
small mononuclear, 73
transition forms, 75
variations in number of, 85
Leucocytic crystals, 364
Leucocytosis (*see* Hyperleucocytosis), 85
Leucopenia, 85, 96
Leukæmia, lymphatic, 107
myelogenous, 109
Lieben's test for acetone, 570
Lientery, 289
Lipacidæmia, 50
Lipaciduria, 577
Lipæmia, 50
Lipuria, 578, 599
Lochia, 668
alba, 668
rubra, 668
Löffler's bacillus, 206
methylene-blue solution, 206
Lohnstein's saccharimeter, 527
Löwy's method of estimating the alkalinity of the blood, 24
Ludwig-Salkowski's method of estimating uric acid, 453
Lymphocytes, 73
Lymphocytosis, 75, 105
Lymphopenia, 75, 107

MACROCYTES, 54
Macrocythæmia, 55
Magnesia mixture, 401
soaps of, in the urine, 598
Magnesium phosphate, 593, 600
Malaria, plasmodium of, 182
Malta fever, bacillus of, 180
Maltose in the urine, 531
Mammary secretion, 672
Marcano's fluid, 142
Marrow cells, 82
Marshall's ureometer, 432
Marsh gas in the gastric contents, 256
Martius and Lüttke's method of estimating hydrochloric acid, 232
Masons' lung (*see* Siderosis), 365
Mast cells, 80
clinical variations of, 108
stains for, 136
Meconium, 334
Medico-legal test for blood, 39
Megaloblasts, 69
Megalocytes, 54
Megastoma entericum, in the feces, 303
in the gastric contents, 263
Melanæmia, 554
Melanin in the urine, 554
tests for, 554
Melanogen, 554
Membranous dysmenorrhœa, vaginal discharge in, 669
Meningeal fluid, examination of, 649
Menstruation, vaginal discharge in, 668
Metalbumin in ovarian cysts, 657
Methæmoglobin, 40
sulphide, 38
Methæmoglobinæmia, 37, 40
Methæmoglobinuria, 489
Methane (*see* Marsh gas), 256
Methylene azure, 137
Mett's method of estimating pepsin, 238
Michaelis' stain, 134
Michaelis and Wolff's stain, 129
Microblasts, 70
Micrococci in pus, 642
Micrococcus ureæ, 624
Microcytes, 54
Microcythæmia, 55
Micro-organisms, in pus, 642
in the feces, 276, 323
in the milk, 675
in the mouth, 200
in the nasal secretion, 336
in the urine, 626
in vaginal discharges, 667
Microscopical examination of cystic fluids, 658
of exudates, 635
of the blood, 54
of the buccal secretion, 200
of the feces, 272, 293
of the gastric contents, 264
of the nasal secretion, 336
of the sputum, 346
of the urine, 585
of the vaginal secretion, 666
of the vomitus, 262
of transudates, 634
Miescher's hæmometer, 155
Milk, 672
chemical composition of, 673
cows', 673

Milk, examination of, 677
fat in, estimation of, 677
human, 673
in disease, 674
proteids of, 678
secretion of, in the adult female, 672
in the newly born, 672
specific gravity of, 673
witches', 672
Milk-curdling ferment in the gastric juice, 239
Millon's reagent, 502
Mohr's test for hydrochloric acid, 228
Monadina in the feces, 300
Monera in the feces, 298
Monocalcium phosphate, 592
Moro's bacillus, 329
Motor power of stomach, examination of, 266
Leube's method, 267
salol test of Ewald and Sievers, 267
Moulds in the urine, 628
Mouth, actinomycosis of, 203
secretions of, 198
tuberculosis of, 203
Mucin in the feces, 333
in the urine, 491
test for, 505
Mucous corpuscles in the urine, 369
cylinders in the feces, 290
in the urine, 618
Mucus in the feces, 290, 296
in the gastric contents, 260
Müller-Weber test for blood, 261
Murexid test, 449
Myelæmia, 86, 109
Myelin granules in the sputum, 349
Myelocytes, basophilic, 83
eosinophilic, 82
neutrophilic, 82
Myelocytosis, 86, 109

NASAL catarrh, 336
secretion, 336
cerebrospinal fluid in, 336
characteristics of, 336
Charcot - Leyden crystals in, 337
in disease, 336
Neisser, gonococcus of, 644
Nematodes, 315
Nessler's reagent, 250
Neubauer's method of estimating oxalic acid, 470
Neusser's granules, 78
Neutral dyes, 121
phosphate of calcium in the urine, 593
red stain for gonococci, 645
sulphur in urine, 411
Neutrophilic leucocytes, 77
Nitric acid test for albumin, 493
Nitric oxide hæmoglobin, 38
Nitrites in the saliva, 199
Nitrogen in the urine, 418
estimation of, 435
according to Kjeldahl, 435
according to Will-Varrentrapp, 437
Nitrogenous equilibrium, 418
Nitro-prusside of sodium as a test for acetone (*see* Legal's test), 569
Nocht-Romanowsky stain, 127
Normal urobilin, 535
Normoblasts, 68
Nose, secretion from, 336
Nubecula in the urine, 369
Nucleated red corpuscles, 67
Nucleo-albumin in the blood, 43
in the urine, 491
test for, 505
Nucleohiston in the urine, 492
Nummular sputum, 341
Nylander's test for sugar, 518

OBERMAYER'S reagent, 540
Obermeier, spirochæta of, 179
Oïdium albicans, 204, 363
Olefiant gas, 257
Oligochromæmia, 33
Oligocythæmia, 34, 59
Oliguria, 375
Oliver's hæmoglobinometer, 155
Orcin test for pentoses, 532
Organic acids in the blood, 50
in the gastric juice, 253
quantitative estimation of, 255
in the sputum, 363
Organized sediments of the urine, 602
Ott's test, 506
Ovarian cysts, 654
Oxalate of calcium crystals in the sputum, 363
in the urine, 590
Oxalic acid, diathesis, 466
in the urine, 466
properties of, 469
quantitative estimation of, 470
tests for, 469
Oxaluria idiopathica, 468
Oxaluric acid, 466
Oxyamygdalic acid, 577
Oxybutyric acid, β, in the urine, 574
estimation of, 575
Oxyhæmoglobin, 18, 32
Oxyphilic leucocytes, 79
Oxyuris vermicularis, 316
Ozæna, 337

PACINI'S fluid, 142
Pancreatic cysts, 660
trypsin in, 660
juice in the gastric contents, 261
Pappenheim's methyl-green-pyronin, 136

Pappenheim's stain for tubercle bacilli, 357
tri-acid stains, 126
Paramœcium coli, 304
Paramucin, 657
Parasites in the blood, 182
in the feces, 275, 297, 323
in the gastric contents, 263
in the sputum, 352
in the urine, 623
malarial, 182
Paratyphoid fever, bacilli of, 170, 328
Paraxanthin in the urine, 442, 455
Patein's albumin, 485
test for, 499
Pathological acetonuria, 568
albuminuria, 472
glucosuria, 508
urobilin, 535, 551
Pentoses in the urine, 532
tests for, 532
Pepsin in the gastric juice, 236
estimation of, 238
tests for, 238
Pepsinogen in the gastric juice, 236
estimation of, 239
tests for, 238
Peptones in the blood, 44
in the feces, 333
in the urine, 489
test for, 504
Peptonuria, 489
Persistent glucosuria, 514
Petrone's fluid, 142
Pettenkofer's test, 283
Phagocytes, 72
Phagocytosis, 72, 190
Pharyngomycosis leptothrica, 205
Phenol, 216, 555, 566
estimation of, 567
in the feces, 279
in the urine, 555, 566
tests for, 216, 566
Phenylglucosazon, 520
Phenylhydrazin test for sugar, 520
Phloroglucin test for pentoses, 533
vanillin test for hydrochloric acid, 226
Phosphates in the urine, 395
estimation of, 401
removal of, from urine, 404
separate estimation of alkaline and earthy, 404
tests for, 400
Phosphatic diabetes, 398
sediments in the urine, 591
Picric acid test for albumin, 493
Pigments in the feces, 283
in the urine, 534
Piorkowski's method, 326
Piria's test for tyrosin, 597
Placenta sanguinis, 28
Plague bacillus, 362
Plaques, 112
enumeration of, 147
Plasma of the blood, 17, 24
Plasmodium malariæ, 182
crescentic bodies, 187
flagellate bodies, 188
hyaline bodies, 183
ovoid bodies, 187
pigmented extracellular bodies, 188
intracellular bodies, 184
segmenting bodies, 186
spherical bodies, 187
staining of, 183
Platodes, 297
Plehn's stain, 137
Pneumoconioses, 365
Pneumonia, diplococcus of, 360
in the blood, 171
sputum in, 360
Poikilocytes, 55
Poikilocytosis, 55
Polarimeter, 528
Pole bacillus, 361
Polychromasia, 62
Polychromatophilic degeneration, 62
Polycythæmia, 58
Polymastigina, 301
Polyuria, 372
Propepsin, 236
Prostatic fluid, 661
Proteids, formed in the stomach, 242
of the blood, 43
reaction of, 244
Proteus vulgaris, 330
Protozoa, 298
in pus, 643
in the blood, 182
in the feces, 298
in the sputum, 352
in the urine, 623
Pseudocasts, 618
Pseudogonococci, 646
Pseudolymphocytes, 83
Pseudomucin, 657
Psorospermiasis, 305
Ptomains in the feces, 334
in the gastric contents 258
in the urine, 581
isolation of, 583
Ptyalin, 199
test for, 199
Purin, 442
bases, 442, 455
Purulent exudates, 640
Pus, 640
chemistry of, 640
corpuscles, in the urine, 605
enumeration of, 609
crystals in, 643
detritus in, 642
general characteristics of, 640
giant corpuscles in, 642

Pus, gonorrhœal, 644
in the feces, 296
in the gastric contents, 262
in the urine, 605
leucocytes in, 641
microscopical examination of, 641
organisms in the urine, 625
parasites in, 642
protozoa in, 643
red corpuscles in, 642
tests for, 606
Putrescin, 582
Pyogenic albumosuria, 486
Pyrocatechin in the urine, 567
Pyrocatechuic acid, 555
Pyroplasma hominis, 192
Pyuria, 605

QUEVENNE'S lactodensimeter, 675

RAY fungus, 362
Reach's test, 269
Red blood-corpuscles, 17, 54
anæmic degeneration of, 62
behavior toward anilin dyes, 62
enumeration of, 139
granular degeneration of, 65
nucleated forms, 67
variations in color, 56
in form, 55
in number, 58
in size, 54
Relapsing fever, spirillum of, 179
Renal albuminuria, 477, 484
Resorcin test, 227
Resorptive power of the stomach, examination of, 268
Reuter's stain, 129
Reynolds' test for acetone, 570
Rhamnose in urine, 532
Rhizopoda, 298
Romanowsky method, of staining, 127
Rosenbach's reaction, 544
test for bile-pigments, 550
Round worms, 315
Roy's method of determining the specific gravity of the blood, 18
Rust-colored expectoration, 340

SACCHARIMETER of Einhorn, 519
of Lohnstein, 527
of Soleil-Ventzke, 528
Saccharomyces cerevisiæ. *See* Yeast.
Saliva, 198
chemistry of, 198
general characteristics of, 198
in special diseases of the mouth, 203
in the gastric contents, 261
microscopical examination of, 200
nitrites in, 199
pathological alterations of, 202
Saliva, ptyalin in, 199
salivary corpuscles in, 200
test for nitrites, 199
for ptyalin, 199
for sulphocyanides, 199
Salivation, 202
Salkowski's method of estimating oxalic acid in urine, 471
xanthin-bases in urine, 456
test for albumoses, 502
for phenol, 566
Salkowski-Neubauer method of estimating the chlorides in urine, 395
Salkowski-Volhard method of estimating the chlorides in urine, 390
Salol test of Ewald and Sievers, 267
Sanarelli's Bacillus icteroïdes, 180
Sand, intestinal, 292
Sarcina pulmonalis, 363
urinæ, 628
ventriculi, 265
Scarlatina, pharyngeal secretion in, 209
Schauffler's methylene-blue-pyronin, 207
Scherer's test for leucin, 597
Schistosoma, 195
Schizomycetes in the feces, 276
Schlösing's method of estimating ammonia, 441
Schmalz and Peiper's method of determining the specific gravity of the blood, 19
Schmidt's fecal fermentation test, 293
Secretions of the mouth, 198
Sediments in acid urines, 588
in alkaline urines, 600
urinary, 369, 585
ammonio-magnesium phosphate in, 591
ammonium urate in, 601
amorphous urates in, 589
basic magnesium phosphate in, 593
bilirubin in, 599
brick-dust, 588
calcium carbonate in, 601
oxalate in, 590
sulphate in, 593
cystin in, 594
epithelial cells in, 602
fat in, 599
foreign bodies in, 630
hæmatoidin in, 599
hippuric acid in, 593
indigo in, 601
leucin in, 595
leucocytes in, 605
mode of examination of, 613
monocalcium phosphate in, 592
neutral calcium phosphate in, 593
non-organized, 588
organized, 602
red corpuscles in, 610

Sediments, urinary, soaps of lime and magnesium in, 598
spermatozoa in, 622
tube-casts in, 612
tumor particles in, 630
tyrosin in, 595
urates in, 589, 601
uric acid in, 588
parasites in, animal, 628
vegetable, 623
xanthin in, 598
Seegen-Schneider method of estimating nitrogen, 437
Semen, 661
chemistry of, 661
general characteristics of, 661
microscopical examination of, 662
pathology of, 663
recognition of, in stains, 663
spermatic crystals in, 661
spermatozoa in, 662
Sepsis, organisms in the blood in, 173
Serosamucin, 638
Serous exudates, 634
Serum-albumin in the blood, 28
in the urine, 472
estimation of, 499
tests for, 492
Serum-globulin in the blood, 28
in the urine, 485
estimation of, 502
test for, 502
Shiga's bacillus, 331
Siderosis, 365
Skatol in the feces, 279
tests for, 280
Skatoxyl, 566
sulphate, 566
Sleeping sickness, organism of, 191
Smegma bacillus, 356, 361, 626
Soaps of lime and magnesium in the urine, 598
Sodium chloride in hydatid fluid, 659
Spectroscope, 41
Spermatic crystals, 364
Spermatocystitis, 623
Spermatorrhœa, 623
Spermatozoa in the semen, 662
in the urine, 622
Spermin, 661
Spiegler's reagent, 498
Spirals of Curschmann, 344
Spirillum of relapsing fever, 179
Spirochæta Obermeieri, 179
Sporozoa, 305
Spotted fever, organism of, 192
Sputum, 338
Amœba coli in, 353
amount of, 339
bacteria in, 354
blood in, 348
cheesy particles in, 342
chemistry of, 366
Sputum, color of, 340
concretions in, 346
configuration of, 341
consistence of, 339
crudum, 341
crystals in, 363
Curschmann's spirals in, 344
Distoma pulmonale in, 283
echinococcus in, 345
elastic tissue in, 342, 350
epithelial cells in, 348
fibrinous casts in, 342
foreign bodies in, 346
general characteristics of, 339
globosum, 341
heterogeneous, 341
homogeneous, 341
leucocytes in, 346
macroscopical constituents of, 342
microscopical examination of, 346
nummular, 341
odor of, 340
parasites, animal, in, 352
vegetable, in, 354
specific gravity of, 341
technique in the examination of, 338
Squibb's ureometer, 433
Staining, methods of, 123
principles of, 119
Staphylococcus pyogenes albus, 176
aureus, 175
citreus, 176
Starch, digestion of, 199
solution, 252
Steatorrhœa, 289
Stercobilin, 284
Stercoraceous material in the vomit, 262
Stock's test for acetone, 570
Stokes' fluid, 33
Stomach, motor power of, 266
rate of absorption in, 268
washing, 215
Stomach-tube, 212
contraindications to its use, 213
its introduction, 213
Stomatitis, catarrhal, 203
gonorrhœal, 203
ulcerative, 203
Stools. *See* Feces.
Strauss and Rohnstein's stain, 134
Strauss' test for lactic acid, 249
Streptococcus pyogenes, 176
brevis, 176
conglomeratus, 176
longus, 176
Streptothrix actinomycotica, 362
Strongyloides, 320
Strongylus duodenalis, 317
Stycosis, 366
Succinic acid in hydatid fluid, 659
Sudan stain for fat, 600
Sugar in the blood, 44
in the urine, 517

Sugar in the urine, estimation of, 522
tests for, 517
Sulphanilic acid test. *See* Ehrlich's reaction.
Sulphates in the urine, 404
conjugate, 405
estimation of, 409
mineral, 405
tests for, 408
Sulphocyanides in the saliva, 199
in the urine, 411
Sulphur, neutral, in urine, 411
estimation of, 413
Syphilitic blood test of Justus, 35

TÆNIA africana, 310
canina, 309
cucumerina, 309
diminuta, 309
echinococcus, 352
flavopunctata, 309
madagascariensis, 311
mediocanellata, 306
nana, 308
saginata, 306
solium, 307
Talquist's hæmoglobinometer, 158
Tartar, 204
Tauro-carbaminic acid in urine, 411
Teichmann's crystals, 39
Test-breakfast of Boas, 211
of Ewald and Boas, 211
Test-dinner of Riegel, 211
Test-meal of Salzer, 211
Test-meals, 211
Thecosoma, 195
Thiosulphates in urine, 44
Thoma-Zeiss' hæmocytometer, 139
Thrush, 204
Toison's fluid, 141
Tollen's orcin test, 532
phloroglucin test, 533
Tongue, coating of, 204
Tonsillitis, 205
Tonsils, coating of, 205
Töpfer's method of estimating hydrochloric acid, 230
test for hydrochloric acid, 226, 230
Toxalbumins in the gastric contents, 258
Transitory glucosuria, 513
Transudates, 631
albumin in, 632
chemistry of, 633
coagulation of, 633
general characteristics of, 631
microscopical examination of, 634
specific gravity of, 631
Trematodes, 297
Triacid stain, Ehrlich's, 124
Pappenheim's, 126
Trichina cystica, 192
Trichina sanguinis hominis nocturna, 192
spiralis, 320
Trichloracetic acid test, 497
Trichocephalus dispar, 320
Trichomonads in the feces, 302
in the sputum, 353
in the stomach contents, 263
in the urine, 629
in vaginal discharges, 667
Trichomonas vaginalis, 302, 628, 667
Trichotrachelides, 320
Triple phosphate crystals in the sputum, 363
in the urine, 514, 591
Tripperfäden, 603
Trommer's test, 517
Tropæolin test for hydrochloric acid, 228
Trypanosomiasis, 191
in the blood, 191
in the cerebrospinal fluid, 655
Trypsin, 660
in pancreatic cysts, 660
test for, 660
Tube-casts in the urine, 612
amyloid, 616
blood, 615
clinical significance of, 619
compound hyaline, 615
epithelial, 615
fatty, 616
formation of, 618
granular, 615
hyaline, 614
mode of examination of, 613
pseudo-, 618
pus, 615
staining of, 614
true, 614
waxy, 616
Tubercle bacillus, 354
cultivation of, 359
detection of, 354
in pus, 642
in the blood, 177
in the cerebrospinal fluid, 654
in the feces, 328
in the milk, 675
in the mouth, 203
in the sputum, 354
in the urine, 626
Tuberculosis, bacillus of, 354
Tumor-particles in the gastric contents, 266
in the urine, 630
Typhoid fever, bacillus of, 165, 325
in the blood, 165
in the feces, 325
in the urine, 625
Tyrosin, 595
in the sputum, 363
in the urine, 595
test for, 597

UFFELMANN'S test for lactic acid, 248
Uncinaria Americana, 317
duodenalis, 317
Unna-Tänzer stain, 352
Unorganized sediments in urines, 588
Uræmia, 47
Urates in urinary sediments, 589
Urea in the blood, 47
in the urine, 414
estimation of, 426
isolation of, 425
origin of, 414
properties of, 422
tests for, 423
nitrate, 423
oxalate, 424
Ureometers, 426
Doremus', 428
Green's, 432
Hüfner's, 432
Marshall's, 432
Simon's, 427
Squibb's, 433
Urethritis, gonorrhœal, 644
Uric acid, 442, 588
crystals of, 588
diathesis, 446
estimation of, 449
Folin's method, 450
gravimetric method, 451
Haycraft's method, 452
Hopkins' method, 449
Ludwig-Salkowski's method, 453
in sediments, 588
in the blood, 48
in the urine, 442
properties of, 448
tests for, 449
Urinary cylinders, 612
pigments, 534
sediments, 585
Urine, 368
acetone in, 568
acidity of, 384
albumins in, 472
albumoses in, 486
alkapton in, 555
alloxur-bases in, 442
ammonia in, 439
amount, 371
animal gum in, 533
parasites in, 623
Bence Jones' albumin in, 487
benzoic acid in, 457
bile acids in, 550
pigments in, 548
blood in, 545
carbohydrates in, 508
casts in, 612
chemistry of, 385
chlorides in, 387
cholesterin in, 551
Urine, chromogens in, 537
crotonic acid in, 576
chyle in, 579, 600
color of, 370
consistence of, 371
cystin in, 412, 594
dextrin in, 532
diacetic acid in, 593
Ehrlich's diazo-reaction, 559
benzaldehyde reaction, 564
epithelium in, 602
fat in, 578
fatty acids in, 577
fecal matter in, 630
ferments in, 579
fibrin in, 490
foreign bodies in, 630
gases in, 580
general appearance of, 369
chemical composition of, 385
glucose in, 508
glucuronic acid in, 533
hæmoglobin in, 489
hippuric acid in, 457
histon in, 492
homogentisinic acid in, 555
indican in, 536
inosit in, 534
kreatin in, 460
kreatinin in, 460
kryoscopy in, 585
lactic acid in, 576
lactose in, 531
lævulose in, 531
laiose in, 532
leucocytes in, 605
maltose in, 531
melanin in, 554
microscopical examination of, 585
mineral ash, estimation of, 386
neutral sulphur in, 411
nitrogen in, 418
nubecula in, 369
nucleo-albumin in, 491
nucleohiston in, 492
odor of, 371
organized sediments in, 602
oxalic acid in, 466
oxaluric acid in, 466
oxyamygdalic acid, 577
oxybutyric acid in, 574
parasites in, 623
pentoses in, 532
peptone in, 489
phenol in, 555, 566
phosphates in, 395
pigments in, 534
referable to drugs in, 559
ptomains in, 581
pus in, 605
pyrocatechin in, 567
quantity of, 371
reaction of, 380

Urine, sediments in, 369, 585
serum-albumin in, 472
serum-globulin in, 485
skatoxyl sulphate in, 566
solids in, 380
specific gravity of, 375
spermatozoa in, 622
sugar in, 508
sulphates in, 404
sulphur, neutral, in, 411
tumor-particles in, 630
urea in, 414
uric acid in, 442
urobilin in, 551
urochrome in, 535
uroerythrin in, 536
urohæmatin in, 543
urohæmatoporphyrin in, 546
urorosein in, 544
vegetable parasites in, 626
xanthin-bases in, 442, 455
Urines, blue, 559
green, 559
Urinometers, 378
Urobilin, febrile, 552
identity with stercobilin, 284
normal, 535
pathological, 535, 551
tests for, 553
Gerhardt's, 553
spectroscopic test, 553
Urobilinogen, 551
Urobilinuria, 552
Urochrome, 534
Uroerythrin, 536
Urofuscohæmatin, 545
Urohæmatin, 543
Urohæmatoporphyrin, 546
Uroleucinic acid, 555
Urophain, Heller's, 544
Urorosein, 544
Uroroseinogen, 544
Urorubrohæmatin, 545
Uroxanthinic acid, 555
Urrhodinic acid, 555

VAGINAL blennorrhœa, 668
discharges, 666
bacteria in, 667
during menstruation, 668
following parturition, 668
general description of, 666
in abortion, 670
in gonorrhœa, 669
in membranous dysmenorrhœa, 669
in uterine cancer, 669
Vaginal discharges in vaginitis, 669
in vulvitis, 669
parasites in, 669
reaction of, 667
Vaginitis exfoliativa, 669
Vincent's fusiform bacillus, 205
Vitalli's test for pus, 606
Vomited material, 259
bile in, 261
blood in, 261
food material in, 259
mucus in, 260
odor of, 263
pancreatic juice in, 261
parasites in, 263
pus in, 262
saliva in, 261
stercoraceous material in, 262
Vomitus matutinus, 221, 261
v. Fleischl's hæmometer, 150

WANG'S estimation of indican, 541
Wassiliew's estimation of albumin, 499
Waxy casts, 616
Weigert-Ehrlich stain, 358
Weigert's elastic tissue stain, 351
Westphal's stain, 136
Weyl's test for kreatinin, 462
Whetstone crystals. *See* Uric acid.
White blood-corpuscles. *See* Leucocytes.
Whooping-cough, bacillus of, 361
Widal's serum-test, 166
Williamson's blood-test in diabetes, 46
Will-Varrentrapp's method of estimating nitrogen, 437
Woodward's method of estimating milk-proteids, 677
Worms. *See* Vermes.
Wright's coagulometer, 29
stain, 130

XANTHIN-BASES in the blood, 49
in the feces, 285
in the urine, 442, 455
estimation of, 456
Xanthoproteic reaction, 280
Xylose in urine, 532

YEAST-CELLS in the gastric contents, 264
in the urine, 628
Yellow fever, bacillus of, 180

ZIEHL-NEELSEN stain, 358
Ziemann's stain, 133
Zymogens in the gastric juice, 236

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